Universidade de Aveiro Departamento de Química

Ano 2013/2014

Ilídio Miguel Teixeira Magalhães

Proteoma da matriz do biofilme de uma estirpe de *S. pseudintermedius*

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Tese de Mestrado apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Bioquímica realizada sob a orientação científica da Doutora Ana Coelho, Investigadora Auxiliar do Instituto de Tecnologia Química e Biológica António Lobo Xavier/UNL, da Doutora Constança Pomba, Professora Associada de Medicina Interna, Departamento de Clínica e Hospital Escolar da Faculdade de Medicina Veterinária da Universidade de Lisboa e pelo Doutor Pedro Domingues, Professor Auxiliar do Departamento de Química da Universidade de Aveiro

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Dedico este trabalho às minhas falecidas avós cuja atitude e espírito de sacrifício, me esforço para emular.

O Júri

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Palavras-chave *Staphylococcus pseudintermedius*, MRSP, biofilmes, proteoma, matriz do biofilme, fatores de virulência, proteómica, MALDI-TOF/TOF, GeLC-MS/MS, nanoLC-ESI-Q-TOF

Resumo

Staphylococcus pseudintermedius (S.pseudintermedius) é uma bactéria patogénica oportunista, responsável pela maioria das infeções cutâneas e pós-cirúrgicas em cães. O número de estirpes resistentes a antibióticos β-lactâmicos está a aumentar constituindo actualmente um dos grandes desafios enfrentados pela medicina veterinária. As bactérias mais resistentes ao tratamento são aquelas que produzem biofilme sendo esta capacidade considerada um fator de virulência. Num biofilme, as bactérias estão envoltas numa matriz de substâncias poliméricas extracelulares (SPE), algumas das quais são proteínas.

Tendo por objectivo obter mais informação acerca do biofilme, foi caracterizado o proteoma da matriz do biofilme de uma estirpe bastante virulenta de S. pseudintermedius isolada de um cão com piodermite profunda. Para tal cultivaram-se biofilmes da estirpe de S. pseudintermedius 5819/10 em meio apropriado, separou-se a matriz das suas células bacterianas e avaliou-se as proteínas presentes quanto ao seu conteúdo e complexidade. Posteriormente o proteoma foi separado por electroforese 1D, caracterizado por nanoLC-ESI-Q-TOF e analisado usando ferramentas bioinformáticas

Constatou-se que o proteoma da matriz do biofilme da estirpe 5819/10 de S. pseudintermedius é muito diverso e que 63% das proteinas podem estar relacionadas com a região extracelular do biofilme ou da membrana plasmática na forma de complexos proteicos. Verificou-se também que a maioria das proteínas identificadas possui funções essenciais para a sobrevivência da bactéria mas não foi possível estabelecer uma relação clara entre elas e a formação de biofilmes. Algumas proteínas que se sabe estarem envolvidas na formação de biofilmes foram identificadas, tratam-se principalmente de factores reguladores da formação de biofilme e outros factores de virulência relacionados com a colonização de um hospedeiro a adesão bacteriana a uma superfície. A prevalência de adesinas e a ausência quase total de proteínas envolvidas na síntese de SPEs, forneceu dados que apoiam a hipótese que a matriz do biofilme do S. pseudintermedius 5819/10 seja constituída por células directamente ou indirectamente unidas entre si.

Keywords *S. pseudintermedius*, MRSP, biofilms, biofilm matrix proteome, virulence factos, proteomics, 2D-PAGE, MALDI-TOF/TOF, GeLC-MS/MS, nanoLC-ESI-Q-TOF

Abstract

Staphylococcus pseudintermedius is an opportunistic pathogenic bacterium responsible for most skin and post-surgical infections in dogs. The number of bacterial strains resistant to β-lactam antibiotics is increasing and are the major challenges now faced by veterinary medicine. Bacteria that produce biofilm are more resistant to treatment and thus, the production of this structure is already considered a virulence factor. In a biofilm, bacteria are embedded in a matrix of extracellular polymeric substances (EPS) some of which are proteins.

With the objective to know more of this array element, the characterization of the biofilm matrix proteome (BMP) from a highly virulent *S. pseudintermedius* strain isolated from a dog with severe pyoderma was performed. Biofilm was developed by culturing the *S. pseudintermedius* strain 5819/10 in specific media. The biofilm matrix was then be separated from bacterial cells and evaluated for their protein content and complexity. Finally, the proteome was separated by 1D electrophoresis and characterized by nanoLC-ESI-Q-TOF and analysed using bioinformatics tools.

The BMP of strain *S. pseudintermedius* 5819/10 consisted in a diverse group of proteins, where 63% of the proteins could be related to either the extracellular region or the plasma membrane, as protein complexes, and most of them had functions essential to cell survival. However, it was not possible to establish a clear relation between them and biofilm formation. Proteins known to be involved in biofilm formation consisted mostly of regulator factors of biofilm formation as well as virulence factors of-mainly-bacterial cell adhesion and host colonization. The prevalence of adhesins and the almost total absence of proteins involved in EPS synthesis pointed to a biofilm matrix where cells are directly or indirectly closely glued together to each other.

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Abbreviations

agr	Accessory gene regulator
AIPs	Auto-inducing peptides
Asb-14	Amidosulfobetaine-14
Atl	Bifunctional autolysin
B2GO	BLAST2GO
Bap	Biofilm-associated protein
Bax	Apoptosis regulator Bax
BHI	Brain heart infusion
BMP	Biofilm Matrix Proteins
CID	Collision Induced Dissociation
cidA	Regulator of murein hydrolase and cell death
CWA proteins	Cell wall associated proteins
CW-TAs	Cell wall teichoic acids
D-Ala	D-alanine
DIGE	Difference gel electrophoresis
DNase	Deoxyribonuclease
DTT	Dithiothreitol
Eap	Extracellular adherence protein
EB	Extraction Buffer
Ebp	Elastin binding protein
EC-TAs	Extracellular teichoic acids
eDNA	extracellular DNA
Efb	Extracellular fibrinogen-binding protein
Emp	Extracellular matrix protein-binding protein
ESI	Electrospray ionization
FnBPs	Fibronectin-binding proteins
GeLC	SDS-PAGE coupled with High Resolution Liquid Cromatography
Glc	Glucose
Glc6Ala	Glucose-6-alanine
GlcNAc	N-acetylglucosamine
GO	Gene Ontology
Hlb	β-hemolysin
HPLC	High Permormance Liquid Cromatography
HTH	Helix-turn-helix
IAA	Iodoacetamide
icaADBC	Intercellular adhesion biosynthetic genes
icaR	Intercellular adhesion regulatory gene
ID	Internal diameter
IPG	Immobilized pH gradient

LC	Liquid chromatography
LM	Light Microscopy
LPXTG	Leucine-proline-any aminoacid-threonine-glycine aminoacid sequence
lrg	Regulator of murein hydrolase and cell death
LS-EPS	Loosely-bound EPS
m/z	Mass to charge ratio
MALDI	Matrix-Assisted Laser Desorption/Ionization
MALDI-TOF/TOF	Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight/Time-Of-Flight tandem mass spectrometer
MIC	Minimal inhibitory concentration
MLST	Multi locus sequence typing
MOWSE	Molecular WEight SEarch algorithm
MRSA	Methicillin-resistant *staphylococcus aureus*
MRSP	Methicillin-resistant *staphylococcus pseudintermedius*
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MSCRAMMs	Microbial surface components recognizing adhesive matrix molecules
MSSA	Methicillin susceptible *Staphylococcus aureus*
NAc	N-Acetylglucosamine
NaCl	Sodium chloride
NCBI	National Center for Biotechnology Information
PBP	Penicillin-binding protein
pgaABCD	Poly-N-acetyl glucosamine biosynthetic genes
pI	Isoelectric point
PIA	Polysaccharide intercellular adhesin
PVL	Panton–Valentine leucocidin
Q-TOF	Quadrupole-time-of-flight mass analyser
Rbf	Protein regulator of biofilm formation
RPLC	Reverse Phase High Performance Liquid Cromatography
sarA	Staphylococcal accessory regulator A
Sbi	Immunoglobulin-binding protein
SCCmec	Staphylococcal chromosomal cassette *mec*
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEC_{canine}	Canine type C enterotoxin
SEPP1	Extracellular elastase

SIET	*Staphylococcus pseudintermedius* exfoliative toxin
sigB	Alternative sigma factor B
SpA	Immunoglobulin G-binding protein A; protein A(short version)
spp.	Species
Spx	Global regulator of stress response genes
SrrAB	Respiratory response regulator
SSI	Surgical site infection
ST	Sequence type
TAs	Teichoic acids
TB-EPS	Tightly-bound EPS
WB	Washing Buffer

1.Introduction

1.1 Staphylococci Biofilms

A biofilm is defined as a "microbial derived sessile community characterized by cells that are irreversibly attached to a substratum, interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced and exhibit an altered phenotype with respect to growth rate and gene transcription" (1). Externally, a biofilm can look like a simple but thick aggregate of cells and polymers (2) but in some cases, pillar or mushroom-shaped structures can be formed (2). Inside the biofilm, bacteria live in a complex community, that possess primitive homeostasis, metabolic cooperativity and a primitive circulatory system that provide some accessibility to essential nutrients even in the deepest regions of the biofilm (2).

However, not all the bacteria in the biofilm express the same phenotype (3). This is because there is a gradient of oxygen, nutrients and electron acceptors, which leads to different gene expression throughout the biofilm (3). For instance, cells in the upper and lower layers, respectively more exposed to oxygen and to dissolved nutrients, present a higher metabolic activity metabolic. Most cells though, are dormant and live in an anoxic environment deprived of nutrients (3).

The phenotype that the dormant cells present is responsible for the biofilm bacterial resistance to antimicrobials, that their planktonic counterparts do not have (4). Dormant cells consist mainly in very slow growing cells with a very low metabolic activity and a small percentage of persister cells (3). Slow growing cells are not very susceptible to antimicrobial agents (5). Persister cells are in a metabolically quiescent state and as consequence, they are able to live without producing or consuming substances that are targets of antimicrobial agents (4). Therefore, they possess multidrug resistance and are able to survive the onslaught of bactericidal antibiotics. (4). Once these chemicals are eliminated, persister cells shift to a metabolic active state and continue the infection of the host by the bacterial biofilm (6).

Bacteria living encased in an biofilm matrix, benefit from its ability to sequester and concentrate environmental nutrients such as carbon, nitrogen and phosphate (7). The biofilm matrix may also provide a diffusion barrier to slow down the infiltration of some

antimicrobial agents (8). It has been shown that reactive chlorine species (ex: hypochlorite) present in several antimicrobials may be deactivated in the surface layers of the biofilm before they are able to penetrate the lower layers (9). Antibiotics such as oxacillin, cefotaxime and vancomycin have also shown reduced penetration throughout *Staphylococcus aureus (S. aureus)* and *Staphylococcus epidermidis (S. epidermidis)* biofilms (10). This finding is not universal as an earlier penetration study of vancomycin and rifampin in *S. epidermidis* biofilms, the biofilm matrix acted as a diffusion barrier (11).

Biofilms may also contribute to the persistence of bacteria (12). Micro-colonies may detach and disperse either due to mechanical shear forces provided by the fluid channels or through a genetically programmed response (12). The detached micro-colonies then migrate to uninfected regions of the host, attach and form a new biofilm (12). In the end, biofilms enhance bacterial growth, provide resistance against antimicrobial agents (and the host immune response), and provide a platform for enabling bacterial spread (13).

1.1.1 Biofilm formation as a virulence factor of *Staphylococcus* infections

Biofilm formation is now recognized as an important virulence factor in several staphylococci infections (14). One reason for it, is that the minimal inhibitory concentration (MIC) of several antimicrobials for sessile bacteria within biofilms can be 10 to 1000 times higher than for their planktonic counterparts (15). Thus, to treat them, a larger quantity of antibiotics is necessary and their undesirable secondary effects are more pronounced (15).

Bacterial biofilms may be of particular concern in veterinary orthopedic surgery associated with implants (16) and skin infections (17, 18). For instance, following the placement of an implant, it can rapidly be coated with a host-derived, protein-based conditioning film, which contains receptors that allow for bacterial attachment, initiating the process for biofilm formation and beginning a surgical site infection (SSI) (19). Indeed, the ability of bacteria to form a biofilm has been shown to be a leading cause of persistent SSIs and pyoderma and thus, the presence of a biofilm can greatly impact the ability to treat them (19).

Therapeutic options available to treat biofilm-associated infections are limited, particularly when the strain is resistant to various antibiotics (20). Removal of infected orthopedic devices, with the associated morbidity and treatment costs, may be the only viable

option (21). So, the development of alternative treatment regimens for biofilm-associated infections is needed.

1.1.1.1 *Staphylococcus pseudintermedius* infections: emergence of methicillin resistance

Staphylococcus pseudintermedius is a recently described gram-positive pathogenic bacterium that was first isolated from the lung tissue of a cat (22). It is an opportunistic pathogen andinfects primarily dogs (23, 24) where it is the leading cause of pyoderma and surgical site infections (SSIs) (25). *S. pseudintermedius* can also infect humans (13, 26), mainly due to contact with companion animals (27). Though these infections are still rare, they can be problematic to treat, particularly when the strain is methicillin-resistant (28).

S. pseudintermedius isolates used to be susceptible to β-lactam antibiotics, but methicillin-resistant *S. pseudintermedius* (MRSP) is becoming prevalent (29). In fact, MRSP has been isolated from dogs, cats and humans (29). In a study by multi-locus sequence typing (MLST) involving isolates from different countries and animal species, it has been found identical or closely related sequence types (ST) in several countries on different continents indicating broad geographic dissemination of the most successful clones (26). Also the ST's of clinical human isolates were closely related to commensal canine isolates suggesting zoonotic transmission (26).

Methicillin resistance is conferred by the presence of the *mecA* gene, which encodes the production of an altered penicillin-binding protein (PBP) that has a low affinity for all β-lactam antimicrobials: penicillins, cephalosporins and carbapenems (30). The *mecA* gene is located on the chromosome of the bacterium on a mobile element called the staphylococcal chromosomal cassette *mec* (*SCCmec*) (31). This element can be transferred between staphylococci species resulting in an increased number of resistant bacteria (32).

MRSP strains have also been shown to be resistant to a large variety of antibiotics besides β-lactams (29). For instance, in cats suffering from urinary tract infections, MRSP was found - and proved – to be resistant to not only to beta-lactams, but also to fluoroquinolones, tetracyclines, macrolides, lincosamides and streptogramins, chloramphenicol, trimethoprim, gentamicin, kanamycin, neomycin and streptomycin (29).

Multidrug-resistant MRSP isolates represent a challenge for antimicrobial therapy in veterinary medicine because of the limited treatment options (29). Based on susceptibility results, the most useful systemic antimicrobials may include chloramphenicol, rifampicin, amikacin, clindamycin or minocycline (33). Aggressive topical therapy has been effective as the only treatment in certain cases, but the adverse effects of some of these medications may limit their usefulness (33).

While *in vitro* susceptibility to vancomycin and linezolid is reported by some laboratories, the use of these drugs in animals is strongly discouraged (33). That is because the pressure put on the use of antimicrobials that are important for treatment of serious infections in humans, raises ethical questions and creates the potential for scrutiny and eventually restriction of extra-label drug use in veterinary medicine (29, 33).

Studies on the risk factors associated with MRSP infections in animals are lacking (29), but are urgently needed. Knowledge is key. A good place to start is by looking into biofilm producing strains of *S. pseudintermedius*. Indeed, biofilm formation in *S. pseudintermedius* has not been fully characterized (34) but has been hypothesized as one of the reasons for the emergence of a few successful MRSP clones (35).

Research is under way and there are positive signs already. The experimental anti-biofilm drug DispersinB® has already been proven to be capable of degrading the polysaccharide intercellular adhesin (PIA) of MRSP biofilm matrices in dogs and thus, destroying the biofilm (36). However, not every biofilm contain PIA (37, 38). Consequently, novel anti-biofilm drugs are needed. Along with PIA, the biofilm matrix of staphylococci biofilms contain teichoic acids, proteins and, DNA (39). Of these compounds, proteins are the most diverse group and may provide key targets for anti-biofilm drugs, or even a new drug itself. After all, DispersinB® is an N-acetylglucosaminidase found on *Actinobacillus actinomycetemcomitans* biofilms, where it is responsible for the detachment of cells from biofilms grown attached to a plastic surface (40).

1.1.2 Biofilm formation and dispersion

As opportunistic pathogens, *S. aureus*, *S. epidermidis* and *S. pseudintermedius*, take advantage of a compromised immune system to infect their host (41). For *S. pseudintermedius*, skin damage (41) and immunosuppression caused by surgical procedures (42) are the main reasons for the pyoderma and SSIs mentioned above.

Not much is known about *S.pseudintermedius* mode of biofilm formation, but *S. pseudintermedius* has biochemical pathways similar to *S. aureus* (22) and expresses several adhesins and extracellular toxins and enzymes similar to it (43). As such, the mechanism for biofilm formation and dispersion of *S. aureus* should provide a model for the yet to be described *S. pseudintermedius* biofilm formation and dispersion mechanisms (figures 1 and 2).

In the planktonic mode of living, both the staphylococcal accessory regulator A (*sarA*) and the alternative sigma factor B (*sigB*) genes are upregulated (44-46), while the accessory gene regulator (*agr*) is dowregulated (47). This results in the inhibition of production of thermostable nucleases and proteases that could otherwise inhibit initial biofilm formation (47). *sigB* also inhibits protease production and additionally, stimulates the production of adherence factors such as the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), like clumping factor, fibronectin binding protein A (fnBPA) and coagulase, (44, 45) which covalently bind to hosts matrix proteins (48).

Covalent attachment of bacterial surface proteins is catalyzed by a family of enzymes called sortases like sortase A of *S. aureus* (49). This enzyme recognizes a sequence of amino acid residues called the LPXTG motif (Leu-Pro-X-Thr-Gly) at the C-terminus of the host membrane surface proteins sequences, and then cleaves it between the Gly and Thr residues (49). Following that, it proceeds to catalyze the formation of an amide bond between the carboxyl-group of threonine and the amino-group of the cell-wall peptidoglycan (49). This way, the bacteria becomes attached to a biological surface (49).

The immature biofilm then increases in cell density until a mature biofilm, where attached cells are embedded in an extracellular polymeric matrix (50). This development occurs either in an extracellular DNA (eDNA) dependent (37), a PIA dependent (51) or even in PIA independent manner (38). Once enough bacteria are attached, transient upregulation

of the *sarA* and *agr* genes, downregulates the production of adhesins and upregulates the production of several immunoavoidance factors and toxins that cause damage to the host organism (52).

When the number of cells within the biofilm reaches a certain number and the density throughout the bacterial community of auto-inducing peptides (AIPs) involved in cell-to-cell communication reaches a quorum sensing threshold, expression of *agr* is induced (47). This in turn upregulates the expression of detergent-like peptides, proteases and thermostable nuclease leading to the release of bacterial cells from the mature biofilm in what is called seeding dispersal (53).

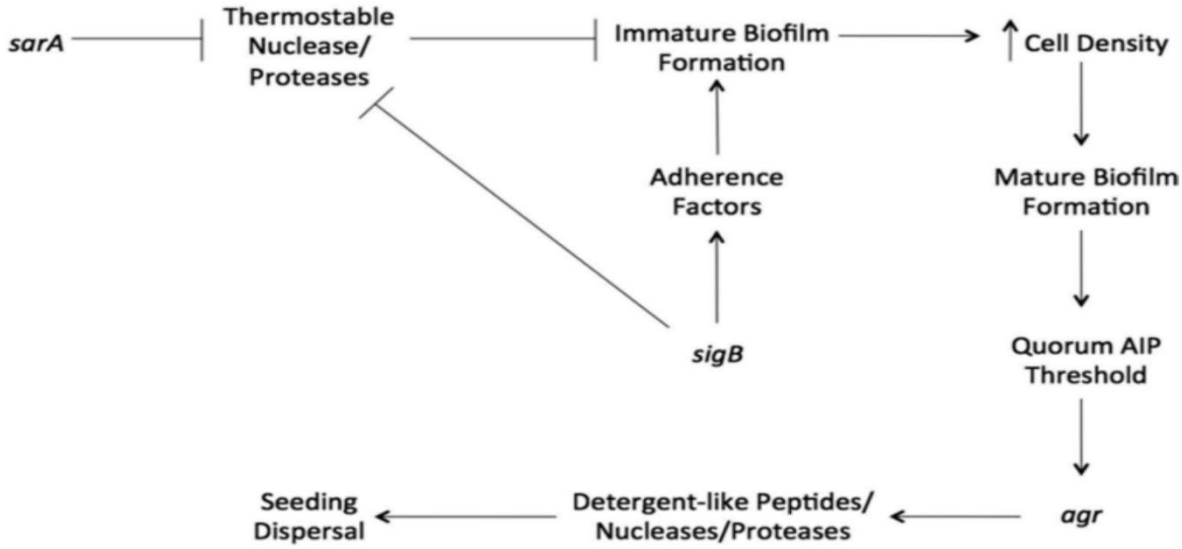

Figure:1 Scheme depicting biofilm formation and dispersion regulated by the agr, sarA and sigB genes, retrieved from reference (54). Pointy arrows heads depicts an event or process that is promoted while hammer-like arrow heads depicts an event/process that is blocked or suppressed. Agr: accessory gene regulator; sarA: staphylococcal accessory regulator A; sigB: alternative sigma factor B.

1.1.2.1 Mechanisms of mature biofilm formation

The development of the biofilm matrix is a key step for the biofilm formation and virulence (14). As mentioned above, it can occur in PIA dependent or independent manner or even in an eDNA dependent mechanism as illustrated in figure 2. *In vitro* biofilm production has also been triggered by the addition of glucose and NaCl to the growth media of *S. aureus* strains and they seem to be associated with different modes of biofilm formation (55). In methicillin-susceptible *S.aureus* (MSSA) isolates, biofilm development in medium containing NaCl was correlated with PIA-dependent biofilm production whereas in MRSA

isolates grown in either medium containing glucose or NaCl, it was PIA-independent and instead was mediated by adhesins (55), which are proteins that glue cells together and to surfaces (56).

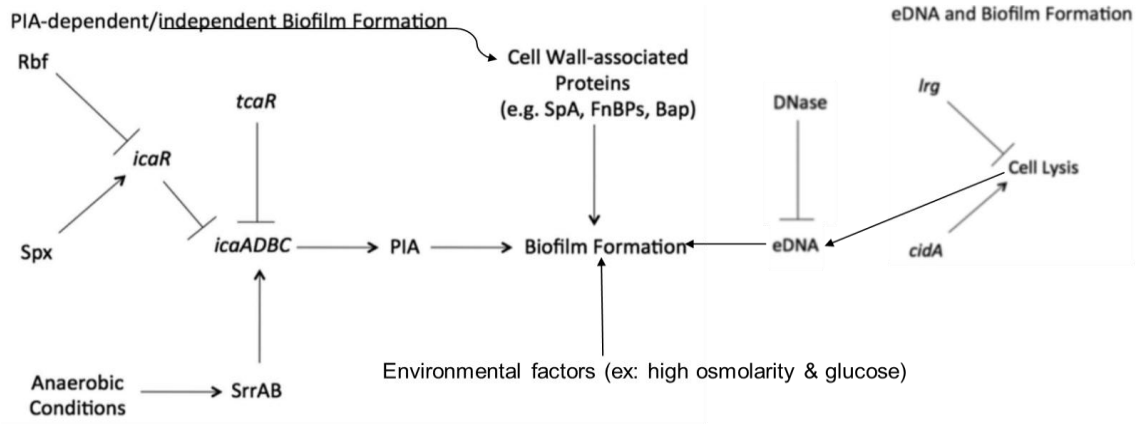

Figure 2- Mechanisms of mature biofilm formation adapted from reference (54). Arrow heads are the same as figure 1.Bap: biofilm-associated protein; cidA: regulator of murein hydrolase and cell death; Dnase: deoxyribonuclease; eDNA: extracellular DNA; FnBPs: fibronectin-binding proteins; icaADBC: intercellular adhesion biosynthetic genes; icaR: intercellular adhesion regulatory genee; lrg: regulator of murein hydrolase and cell death; PIA: polysaccharide intercellular adhesin; Rbf: protein regulator of biofilm formation; SpA: immunoglobulin G-binding protein A; Spx: global regulator of stress response genes; SrrAB: respiratory response regulator.

1.1.2.1.1 Mature Biofilm Formation in PIA-dependent manner

After adhesion, bacteria may start biofilm production through the synthesis of an adhesive polysaccharide polymer known as PIA (51). In *S. aureus*, PIA is produced *in vivo* and *in vitro* from UDP-N-acetylglucosamine via transcription of genes of the intercellular adhesion (*ica*) locus: the biosynthetic *icaADBC* operon (57). These genes are necessary for biofilm formation and virulence and are upregulated in response to anaerobic growth, such as the internal environment of the immature biofilms (58). Under anaerobic conditions, PIA synthesis is triggered by the staphylococcal respiratory response regulator (*SrrAB*) through the binding of a 100 bp DNA sequence upstream of the *icaADBC* operon (59).

The synthesis of PIA can be repressed by the expression of transcriptional regulator of the teicoplanin-associated locus (*tcaR)*, the intercellular adhesion regulator gene (*icaR*) and indirectly, the global regulator of stress response genes (*spx*) (60-62). *icaR* encodes a transcriptional repressor that represses PIA production by binding to the *ica* cluster promoter (60, 61) and *spx* modulates this action (62). However, transcription of *icaR* can be repressed by the protein regulator of biofilm formation (Rbf) (63). When this occurs or when *icaR* is

simply deleted, the expression of the *icaADBC* operon is enhanced, PIA production increases as well as the biofilm formation (60, 61, 63).

1.1.2.1.2 Mature Biofilm Formation in PIA-independent manner

Despite the importance of the *ica* gene locus in biofilm development, biofilms can occur in an ica-independent fashion (55). In *ica*-deletion mutants of MRSA, PIA-independent biofilm formation can be mediated by cell-to-cell adhesion through their cell wall-associated proteins (55). Proteins arbitrating this type of biofilm formation include the *S. aureus* protein A (Spa) (64), fibronectin-binding proteins (FnBPs) (65) and the biofilm-associated protein (Bap) (66).

1.1.2.1.3 Mature Biofilm Formation in eDNA-dependent manner

Extracellular DNA (eDNA) can also induce PIA-independent biofilm formation (37, 67). On the other hand, DNase treatment degrades eDNA and inhibits eDNa-mediated biofilm formation (68). Genomic DNA is the source of eDNA and its release is arbitrated through cell lysis and controlled by *lrg* and *cidA* genes expression (37, 67). *lrg* is a regulator of murein hydrolase and cell death (37) while *cidA* is an holin homolog involved in cell lysis and encodes an effector of murein hydrolase activity and also regulates cell death (67). Upregulation of the *lrg*, results in inhibition of cellular lysis, DNA release and biofilm formation (37). On the other hand, *cidA* gene expression enhances cellular lysis, DNA release and biofilm formation (67).

1.1.3 Constitution of the Biofilm Matrix

1.1.3.1 Polysaccharides

In staphylococci biofilms there are two main classes of polysaccharides: the polysaccharide intercellular adhesin (PIA) and the extracellular teichoic acids (EC-TAs) (51). PIA is a linear β-(1,6)-linked N-acetylglucosaminoglycan containing about 130 N-acetylglucosamine (GlcNAc) residues, partially substituted with O-succinyl groups and partially de-N-acetylated (69) and its monomeric structure is presented in figure 3. As for the EC-TAs, they are composed of glycerol, phosphate, glucose, and GlcNAc in *S. epidermidis* (70) and phosphate, ribitol, glycerol, GlcNAc, and $_{D}$-Ala in *S. aureus* (71).

EC-TA are highly polar and hydrophilic molecules, whereas PIA is rich in relatively hydrophobic NAc groups (51). Both groups of molecules have positive and negative charges due to substitution of hydroxyl with charged groups: free amino-groups and O-succinyl substituents in PIA, D-alanyl esters and phosphate groups in EC-TA (51). The amount of substitutions may vary and be influenced by the growth conditions (70). Also, the capacity to regulate the number of positive and negative charges, as well as the hydrophilic properties of its biofilm constituents by staphylococci, should increase its ability to form biofilm on surfaces with different physicochemical properties and to survive and proliferate under varying environmental conditions (51).

PIA was first characterized in *S. epidermidis* (69) and subsequent studies in the model strain *S. epidermidis RP62A* (70) have revealed the presence of a similar polymer, where only the degree of N-deacetylation and O-succinylation as well as phosphorylation varied between them. PIA not only promotes intercellular adhesion and biofilm formation (72), it also contributes to the pathogenesis of biomaterial-associated infections , to the binding to hydrophilic surfaces and to hemagglutination (73).

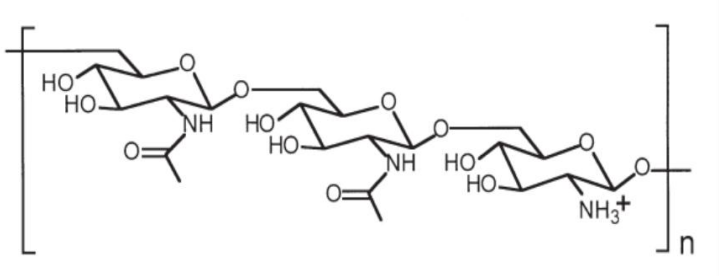

Figure 3- PIA monomer consisting of a partially de-N-acetylated β-(1,6)-linked N-acetylglucosaminoglycan. Image based on the data from reference (69), but retrieved from reference (72).

PIA or similar polymers seem to be wide spread in bacterial biofilms (51). In Gram-negative bacteria *Escherichia coli*, the gene locus *pgaABCD* is responsible for the production of poly-β-(1,6)-GlcNAc (PGA) (74), which is a polymer with a structure very similar to PIA (74). Homologues of *pgaABCD* were found in other Gram-negative bacteria such as, *Actinobacillus pleuropneumoniae* and *Aggregatibacter actinomycetemcomitans* (75, 76). What is remarkable is that coding genes for PIA and PGA have a low degree of homology: *pga* and *ica* genes do not have a great degree of homology, but the polymers they encode are structuraly and functionally the same. (74).

Teichoic acids (TAs) are an integral part of the cell wall of staphylococci bacteria (51). Due to their alanine content (and the charge its positively amino group brings), cell wall teichoic acids (CW-TAs) contribute to the formation of *S. aureus* biofilms and enhances the adhesion of *S. epidermidis* cells to fibronectin-coated surfaces in the early stages of biofilm formation (77). TAs, are also found beyond the cell wall and can be secreted to the biofilm matrix, EC-TAs (78). In fact, due to their cell wall origin, the composition and structure of both CW-TAs and EC-TAs in *S. epidermidis* RP62A and *S. aureus* MN8M are the same (71, 79).

The EC-TA of *S. epidermidis* is composed of (1,3)-linked poly(glycerol phosphate), substituted at the position 2 of the glycerol residues with α-Glc, α-GlcNAc, D-Ala, or α-Glc6Ala (79). In *S. aureus* however, there is also a poly(ribitol phosphate) EC-TA polymer (71). In the poly(ribitol phosphate) chain, nearly 100% of ribitol is substituted with β-GlcNAc at position 4 and its structure matches the one described for *S. aureus* H (80). As in *S. epidermidis* polymer, the glycerol residues are (1,3)-linked, but most are not substituted: only about 20% are acylated with D-Ala at position 2 (71). Looking into these evidences, the degree of substitution in D-Ala, an important pathogenic element (51), seems different in both bacteria species and one could expect similar differences for the *S. pseudintermedius* biofilm matrix .

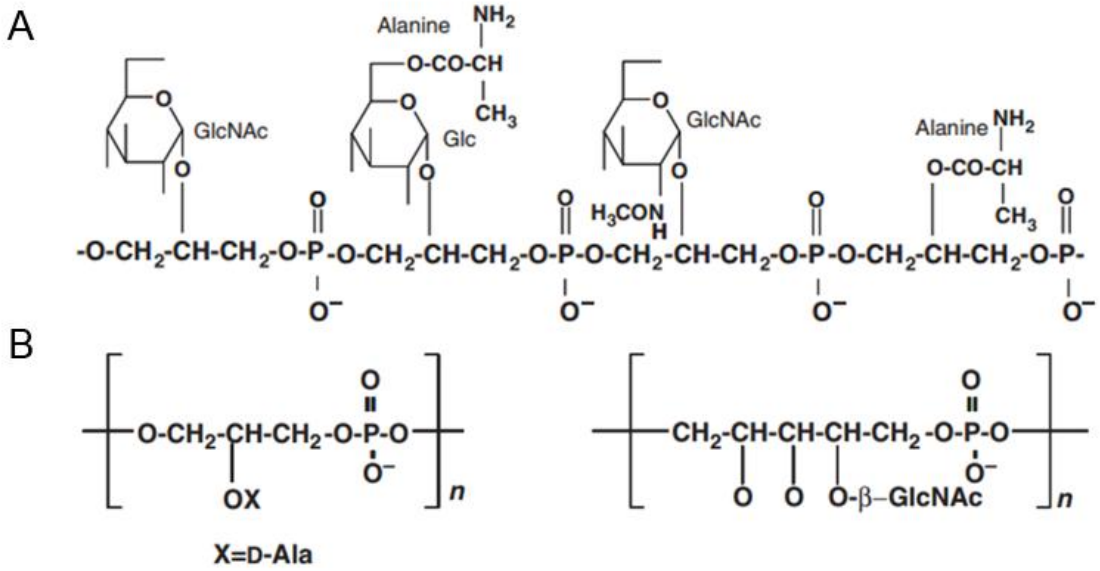

Figure 4- EC-TA monomers of S. epidermidis RP62A (A) and S. aureus MN8m (B). Image based on the data from references (71, 79) but retrieved from reference (51) for the S. pseudintermedius biofilm matrix.

1.1.4.2 eDNA

Extracellular DNA (eDNA) is considered a major structural component of the biofilm matrix of *S. aureus*, whereas it is only a minor component of biofilms formed by *S. epidermidis* (81). That is because when treated with DNase I, the formation of *S. aureus* biofilms is inhibited and detachment of pre-formed *S. aureus* biofilms occurs (81). The eDNA of *S. aureus* consists of small fragments of genomic DNA (11 kb) and they mediate intercellular adhesion (81). This genomic DNA is obtained through a phenomenon analogous to programmed cell death and is regulated by the *cidA* gene (82). This release, also promotes biofilm formation and growth (82), as previously described.

1.1.4.3 Proteins

There are many known proteins within the biofilm matrix (reviewed in (39)), but regarding staphylococci, not many of them were identified. In table 1, secreted proteins found on the biofilm matrix of *S. epidermidis* and *S. aureus* strains are presented (56, 83). They were identified in two recent studies and provide clues about which proteins might be present in the *S. pseudintermedius* biofilm matrix. The secreted proteins identified comprise binding proteins, enzymes, and toxins.

Table 1 Secreted proteins from S.aureus (56) and S.epidermidis (83) found exclusively in their biofilm matrices

Secreted Proteins	Protein name & abbreviation	Acession Number |Entry Name	Gene	Extracellular function	Bacteria
Enzymes	Extracellular elastase, SEPP1	P0C0Q3|SEPA_STAEP	*sepA*	Hydrolysis of casein and elastin and glucagon	*S.epidermidis*
	Epidermin leader peptide processing serine protease EpiP	P30199|EPIP_STAEP	*epiP*	Complement evasion	
	Glutamyl endopeptidase	P0C0Q2|GSEA_STAES	*gseA*	Human fibronectin and type 1 collagen hydrolysis	

	Thermonuclease	Q5HHM4 NUC_STAAC |	*nuc*	Biofilm dispersion: eDNA hydrolysis	*S.aureus*
	Staphylocoagulase	Q5HJE9 |Q5HJE9_STAAC	*SACOL0209*	Adhesion to fibrinogen and its conversion into fibrin; blood clotting	
	Bifunctional autolysin, Atl	Q99V41| ATL_STAAN	*atl*	Biofilm formation; adhesion	
Binding Proteins	Fibrinogen-binding protein, Efb	A6QG59| FIB_STAAE	*fib*	Biofilm formation, adhesion, complement evasion	
	Extracellular adherence protein, Eap	Q53599| MAP1_STAAU	*map*	Biofilm formation, cell-to-cell adhesion	
	Immunoglobulin-binding protein, Sbi	Q99RL2| SBI_STAAN	*sbi*	Complement evasion	
	Immunoglobulin G-binding protein A, SpA	A6QD95| A6QD95_STAAE	*spa*	Biofilm formation, complement evasion	
	Extracellular matrix protein binding protein, Emp	Q99VJ2| EMP_STAAM	*emp*	Biofilm formation, adhesion, abscess formation	
Toxins	β-hemolysin, Hlb	Q931I6| Q931I6_STAAM	*truncated-hlb*	Biofilm formation, sphingomyelinase activity	
	Uncharacterized leukocidin-like protein 2	Q5HEH9| LUKL2_STAAC	*SACOL2006*	Bax-independent apoptosis of human neutrophils	

The binding proteins were identified in the *S. aureus* biofilms (56). Some of them are immunoglobulin binding proteins: Protein A (SpA) and Sbi (56). These proteins bind to human IgG - inactivating it - and thus evading the complement system, "an elaborate network of cascades for dealing with microbial intruders" (84). Moreover, SpA has a pivotal role in PIA-independent biofilm formation by inducing bacterial cell aggregation and thus acting as an adhesin (64).

The other binding proteins found were the adhesins Eap, Efb and Emp (56). Eap was shown to stimulate cell aggregation and biofilm formation and also strongly bound itself to the *S. aureus* cell surface (56). It was also able to form biofilm matrix architecture and contribute to biofilm development through the formation of a structural framework in the biofilm matrix (56). Efb promotes biofilm formation and complement system evasion through the stimulation of fibrinogen binding to ADP-activated platelets and inhibition of platelet aggregation, respectively (85). At last, Emp adheres to the host fibronectin, fibrinogen, and vitronectin (86), plays an important role in low-iron-induced biofilm formation of *S. aureus* (87) and is necessary for abscess formation (88).

Enzymes secreted by *S. aureus* onto its biofilm matrix were autolysin, coagulase and thermonuclease (56). Autolysin is required for both FnBP- and PIA-mediated biofilm development on hydrophobic polystyrene and to the attachment to hydrophilic polystyrene (65). Coagulase adheres to fibrinogen and catalyzes its conversion into fibrin, leading to the formation of blood clots while thermonuclease has been implicated in bacterial cell detachment, through the hydrolysis of eDNA (37).

Enzymes secreted onto *S. epidermidis* biofilm matrix on the other hand, were the tissue-damaging proteases serine protease EpiP, glutamyl endopeptidase and extracellular elastase (83). These enzymes play a key role in the inactivation of the host defense mechanisms and thus in the persistence of *S. epidermidis* biofilms and infections (83). Serine protease EpiP is involved in complement evasion through the processing of epidermin, a lantibiotic that helps to exclude competing organisms that are sensitive to its bactericidal activit (89). Glutamyl endopeptidase, also known as serine proteinase, hydrolyses human fibronectin and type 1 collagen (90). Finally, extracellular elastase is a metalloprotease that has a low substrate specificity and hydrolyzes casein, elastin and glucagon (91).

A couple of toxins were also found on the biofilm matrix of *S. aureus*, namely a hemolysin and a leukotoxin. β-hemolysin has sphingomyelinase activity with a high affinity for sphingomyelin (92). This toxin is also capable of forming covalent cross-links to itself in the presence of eDNA producing an insoluble nucleoprotein matrix *in vitro* and, thus forming a biofilm. The leukocidin has a molecular mass close to 40 kDa, it may be one of the two proteins that constitute the *S. aureus* Panton-Valentine leukocidin (93) that directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils (94).

In spite of large variety of secreted proteins in both biofilm matrixes, the overall number of those only present in this proteome was very low (56, 83). The biofilm matrix proteome (BMP) of *S. aureus* was comprised of only 14% secreted proteins but more than 50% were already known as cytoplasmatic proteins (56). Among these, there were homologs of enzymes with functionality related to protein synthesis and carbohydrate metabolism also found on *E. coli* biofilm matrix, such as ribosomal proteins (30S and 50S) and glyceraldehyde-3 phosphate dehydrogenase, respectively (95). In the *S. epidermidis* BMP, the secreted proteins only accounted for 16% of BMP, with the rest being cell wall associated proteins (CWA proteins) (83).

All of this evidence provides valuable information that gives some clues to predict which proteins might be found on the biofilm matrix of *S. pseudintermedius* (figure 5). Similar to *S. aureus*, *S. pseudintermedius* produces a variety of virulence factors, including enzymes such as coagulase, thermonuclease and various proteases, surface proteins like clumping factor and protein A, and toxins such as cytotoxins, exfoliative toxins and enterotoxins (reviewed in (96)) which could also be found on the biofilm matrix . Of note are the canine type C enterotoxin (SEC_{canine}), a superantigen, and the *S. pseudintermedius* exfoliative toxin (SIET), as both have been associated with canine pyoderma infections (97, 98).

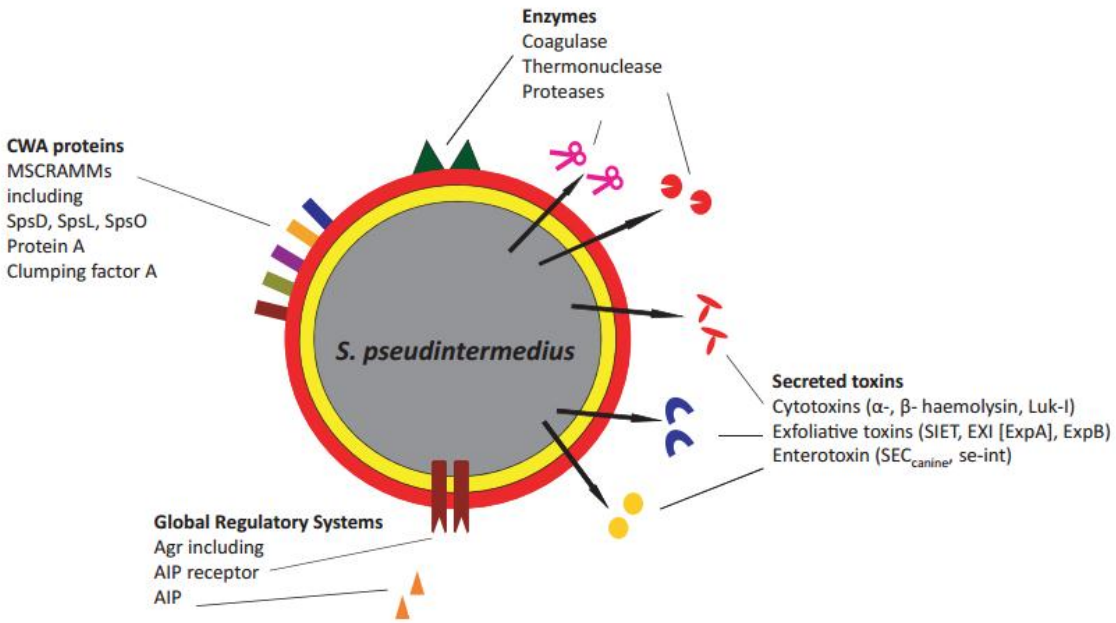

Figure 5- Main secreted and cell-wall associated proteins of s.pseudintermedius that may be found on its biofilm matrix. Image retrieved from reference (96).

1.2 Experimental methods for the identification of staphylococci biofilm matrix proteins

In order to characterize biofilm matrix proteins, several steps must be accomplished: biofilm formation, biofilm matrix and protein extraction protein separation and identification. *In vitro* biofilm formation is performed by cultivating staphylococci in tryptic soy broth (TSB) and brain heart infusion (BHI), supplemented with either glucose of NaCl within plastic flasks with a large surface area to maximize the adhesion (99). Then, the biofilm matrix is extracted using a cation exchange resin (83, 100) or concentrated NaCl (56). Proteins are then isolated through trichloroacetic acid precipitation (101) and then ressolubilized in an electrophoresis running buffer, in order to get simplified protein fraction to proceed with the proteomic analysis.

Proteins are usually then separated by polyacrylamide gel electrophoresis, namely two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) (102). There is also an alternative procedure to 2-DE-PAGE, where sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is combined with High Performance Liquid Cromatography after tryptic digestion of each band and it is called GeLC-MS.

1.2.1 SDS-PAGE and 2D-PAGE for protein separation

The SDS-PAGE is a separation method where proteins are separated according to their molecular mass (103). Protein samples are denatured with SDS in order to obtain their linear primary amino acid structure (104, 105). After that, they are transferred to a porous polyacrylamide gel and subjected to an electric current (104, 105). SDS gives proteins an even negative charge and so, they will move towards the positive pole at velocity dependent on their molecular mass (105). Those with higher mass are the slowest and also the most difficult to migrate through the gel (105).

The pore size in the gel is crucial for a good separation (105). The smaller the pore, the harder it will be for bigger proteins to penetrate and move through the gel (105). On the other hand, if the pore size is too large, proteins with a similar mass may migrate at the same time and will not separate themselves (105). The key to regulate the pore size is the acrylamide percentage on the gel, as the pore size is inversely proportional to the amount of

acrylamide used (105). As a reference point, in the *S. epidermidis* proteomic study mentioned in 1.1.4.3, a 12% polyacrylamide gel was used (83).

Proteins separated by SDS-PAGE subsequently rely on mass spectrometry (MS) techniques for identification and further characterization (104). Protein in-gel are excised and then *in gel* digested with a specific protease, namely trypsin (104). The mass of the resulting tryptic peptides and of their fragments is determined using a mass spectrometer. These results are combined in order to obtain protein identifications (104).

1.2.2 MALDI- TOF/TOF mass spectrometry for protein identification

Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight/Time-Of-Flight tandem mass spectrometer (MALDI-TOF/TOF) is an instrument with the advantages of high sensitivity for peptide analysis and comprehensive fragmentation information provided by high-energy collision-induced dissociation (CID) (106). MALDI ionizers create mainly single charged ions (107). Samples are incorporated into the crystalline structure of small UV radiation absorbing molecules, that provide a vehicle for ions to be created from polar or charged biomolecules (108). When laser radiation strikes the matrix crystals, the energy deposition is thought to cause rapid heating of the crystals brought about by matrix molecules emitting absorbed energy in the form of heat (108). The rapid heating causes sublimation of the matrix crystals and expansion of the matrix and sample into the gas phase (109). Ions may be formed through gas phase proton-transfer reactions in the expanding gas phase plume with photo-ionized matrix molecules (109).

MALDI ionizers are usually coupled to TOF analyzers (110). Within this device, mass-to-charge ratios are determined by measuring the time it takes for ions to move through a field-free region (110). Given a constant accelerating voltage, the flight time for an ion will be related to its mass to charge ratio (*m/z*) (110).

The flight path for an ion can be increased, without increasing the size of the flight tube through the incorporation of an ion mirror or reflectron at the end of the tube (110). With this device in place, ion direction is reversed to send the ions back down the same vacuum chamber at a slightly different angle so the flight path of the reflected ions does not cross with the ions entering the reflectron (110). The reflectron can also correct minor kinetic energy differences among ions of the same *m/z* value and so minimize variations in their

fight times (110). Ions with higher kinetic energy arrive earlier at the reflectron but also penetrate deeper before reflection than the less energetic ions (110). Thanks to that, ions with the same *m/z* ratio but different initial energies are capable of meeting at the detector at about the same time (110).

Structural information is deduced from ion fragmentation (110). This is because there can be peptides with the same mass but different composition and amino acid sequence. Thus, incorporating a collision cell and a second TOF to the equipment will allow this type of analysis (106). A precursor ion is fragmented inside the collision chamber and the mass of the fragment ions is determined in the second TOF. The analysis of the generated MS/MS spectrum enable to deduce the peptide amino acid sequence (106).

The MS/MS peak mass values of each ion fragment are then compared with the theoretically expected tryptic peptide masses in databases such as the SWISSPROT or the NCBI non-redundant (111), with the help of a search engine such as Mascot (112), which uses a development of the MOWSE algorithm (112, 113). The program gives to each ion fragment a score based on the probability of the matching between the experimental data and the sequence databases being chance events (112). The identification match with the lowest probability will be the best match, and it will be significant if it has a probability of occurring inferior to 0.05 (112).

1.2.3 GeLC-MS/MS

GeLC-MS is generally considered to be the technique that provides the highest number of protein identifications of the standard proteomics workflows (114). In this technique, proteins are first separated by 1DE (SDS-PAGE) and then each lane of the gel is excised into multiple gel slices (114). Each of these slices is then *in-gel* trypsin digested and provides the peptide samples for a distinct LC-MS analysis (114). This allows overcoming the ion suppression effect common in MS analysis, and increases the number of proteins identified in a complex sample.

Performing an initial 1DE has other advantages. Firstly, the proteins can be solubilized with SDS, assuring maximum solublization compared to other protein separation methods (114). And secondly, the first dimension gel gives insight on the initial apparent molecular mass of the protein identified from its migration on SDS-PAGE (114) and this

can provide insight into protein processing or modification (115). On the other hand, the main problem with this approach is that is very time consuming (114).

1.2.3.1 High resolution liquid chromatographic techniques: HPLC and nano HPLC

Liquid chromatography (LC) can be regarded as the separation of components of a mixture based upon the rates at which they elute from a stationary phase typically over a mobile phase gradient (116). Differing affinities of the mixtures components for the stationary and mobile phase's leads to their separation, since certain components will be more attracted to the mobile phase and will elute quickly whilst others will be retained by the stationary phase for longer and therefore will elute more slowly, i.e. have a larger retention time (RT) (116). HPLC on the other hand is an evolution, where high-pressure pumps and strengthened packed columns are combined to improve separations.

The HPLC device consists of solvent reservoirs, one for each of the mobile phase eluents (solvent A and B), as well as further reservoirs for autosampler syringe and line washes. A high-pressure pump forms the solvent delivery system, which generates and meters a specified flow rate (typically millimeters per minute). A solvent partitioning valve allows the mixing of solvents A and B at specified ratios and time gaps, thus permitting the operator to programme the HPLC to perform gradient elution steps (117).

To commence the HPLC cycle sample analysis, an autosampler injector system introduces the sample to the mobile phase stream and is carried to the HPLC column (116). In the case of reverse phase HPLC (RPLC) for peptide separation, the column is packed typically with silica–$C_{18}H_{37}$ particles to act as a non-polar retaining stationary phase (116). At an appropriate time point during the HPLC separation (which is sample matrix-dependent and must be optimized to allow for the elution of the maximum number of polar sample components), is introduced to the mobile phase in increasing ratios in an incremental manner, thus the increasing organic content of the solvent leads to the elution of the retained non-polar compounds from the stationary phase. (116). Throughout the chromatographic run a mass spectrometer is recording the signal of the eluted sample components, the signal is amplified and delivered to a computer where it is processed and displayed as a chromatogram (116).

Factors influencing the performance of the HPLC system are the polarity of the sample compounds that are being analyzed; the composition of the mobile phase; and the chemical properties of the stationary phase (116). However, there are several, other which influence the efficiency of HPLC separations and were utilized in the development of nano HPLC such as column length and internal diameter and particle size (116).

Here enters nano-LC that offers higher efficiency, shorter analysis time and better compatibility with MS, due to the relatively low flow-rates (40-600 nL min^{-1}) that allow the transfer of the entire effluent from the column into the MS (118). Nano-LC uses capillary columns of 10-100 μm internal diameter (ID) packed with the same type of stationary phase as used in regular HPLC. In nano-LC the use of pre-columns is recommended, since capillaries can be easily blocked at the inlet, when real samples are injected. Pre-columns can also be used for sample clean up and pre-concentration. (119).

Providing that a column bed is uniformly packed and stable, the chromatographic separation efficiency is determined by the particle size and column length (120). Longer lengths of column enhance chromatographic separation, though at the expense of increased analysis times and system back pressure (120). Smaller particle sizes lead to an increased surface area improving compound retention and providing improved chromatographic separation at the expense of an increased pressure being required to drive the mobile phase (121). Unfortunately, decreases in column particle size result in greater system back pressures, as does increases in column length along with increased solvent consumption and sample analysis times (120).

In modern HPLC and nano-LC, particles in the μm range are commonly employed to maximize the chromatographic resolution power along with short column lengths to reduce sample analysis times, backpressure, and solvent consumption (121). The column's ID influences the selectivity of separation and detection sensitivity in gradient elution as well as determining the volume of sample that can be loaded. Narrow-bore columns are commonly used in LC-MS applications since they offer greater sensitivity than the larger-ID columns, although they restrict the volume of sample that can be loaded (117). Finally, a nanospray ionization source is coupled to the nanoLC cromotograph.

1.2.3.2 ESI-MS/MS

1.2.3.2.1 Ionization Source

In the electrospray ionization (ESI) of peptides, an acidic, aqueous peptidic solution is sprayed through a small-diameter needle (122). A high, positive voltage is applied to this needle to produce a Taylor cone from which droplets of the solution are sputtered (122). The positive charged droplets then move from the needle towards the more negatively charged instrument (122). During the course of this movement, evaporation of the droplets occurs, reducing their size (122). This leads to split of the droplets into smaller ones due to high number of positively charged particles (122). The evaporation and droplet-splitting cycle repeats until the small size and charging of the droplet desorbs protonated peptides into the gas-phase, where they can be directed into the mass spectrometer by appropriate electric fields (122).

ESI tends to protonate all available basic sites in analyte molecules (122). In peptides, they are the N-terminal amine moiety and the basic side groups of the lysine, arginine, and histidine residues (122). As a result, multiply protonated peptide ions are observed whenever a lysine, arginine, or histidine residue is present in a peptide (122). Doubly charged peptides tend to predominate in tryptic digests of proteins because of the proteolytic specificity of trypsin (122). This enzyme cleaves amide bonds at the C-terminal side of each lysine and arginine residue (122). In general, the peptides produced have only two basic sites - the N-terminus and the side chain of the C-terminal lysine or arginine residue (122). Consequently, ionization takes place by protonation of those two sites (122). More, highly charged tryptic peptides nearly always contain internal histidine, lysine-proline bonds, arginine-proline bonds, or missed cleavage sites (122). As a result, the maximum charge state of a peptide in a tryptic digest can provide some information about its structure (122).

ESI is a very efficient process for whom the efficiency of protonation of amino acid residues basic sites in acidic environments plays a major role (122). To add to its atratitiveness to proteomic studys, ESI is compatible with reversed-phase high-performance liquid chromatography (RPLC) solvent systems (122). Water/solvent mixtures have excellent spray properties and, although methanol might be preferred, acetonitrile is an acceptable solvent for electrospray (122).

Nanospray is a low-flow spray-ionization technique used in the mass spectrometric analysis of protein digests (122). The fundamental ionization mechanism is same but distinguished by extremely low flow rates from the spray needle (122). The dimensions of the Taylor cone and the sputtered droplets produce the sensitivity enhancement seen with nanospray (123). As the flow rate is lowered, and as the size of the electrospray needle is reduced, the dimensions of the Taylor cone and of the droplets that are produced are also reduced (123). The efficiency of desorption of analytic peptide ions from the electrosprayed droplet increases as the size of the droplets decreases because of the larger surface area of the droplet relative to its total volume (123). As a result, a greater proportion of analyte is desorbed from the droplets and is transmitted from the spray needle to the entrance aperture of the mass spectrometer and detectable signals can be observed with attomole amounts of peptides (123). The result of this sensitivity enhancement is that nanospray ionization extends the sequencing of proteins in electrophoretic gels down to the silver stain-detectable level, which is equivalent to as little as 10 to 100 fmol of protein in the gel (123).

A practical effect of nanospray is that the microliter volumes of sample produced by a protein digest can be sprayed for extended periods (122). Because ions are generated for a longer period, more sophisticated experiments can be performed, such as MS-MS experiments to investigate structure of product ions, or optimization of collision conditions by using a variety of energies, or simply acquiring more product ion spectra than could be acquired when operating on the chromatographic time scale (122). The primary disadvantages of nanospray ionization relate to inherent difficulties of miniaturization; namely the need for microscopes to manipulate and place the needle, obstruction of the needle, and, in particular, the practical problems related to executing in-line HPLC separations at low nanoliter-per-minute flow rates (122). These difficulties, although generally manageable, have been the driving force behind the development of microspray ionization as a compromise between the flow conditions of electrospray ionization and nanospray ionization (122).

Ionization suppression by trifluoroacetic acid is most easily solved by replacing the trifluoroacetic acid in the HPLC buffer systems with acetic acid (122). This step is important to maximize the sensitivity of electrospray ionization because the ion-pairing activity of trifluoroacetic acid tends to disrupt the protonation reaction, which suppresses positive ion generation (122). Trifluoroacetic acid is a standard ion-pairing agent in reversed-phase

HPLC analysis of peptides (122). Although the choice of ion pairing agent can have dramatic effects of the quality of separation in complex mixtures, it is important to remember that good chromatographic separation of peptides can also be accomplished with acetic acid, and the high UV cut-off that makes its use impractical for typical HPLC analyses is irrelevant with mass (122).

The problems associated with the changing proportions of solvent and water in the column effluent are minimized by post-column mixing of the column flow with a sheath liquid designed to optimize analyte signal generation and removed altogether when using nanospray, which does not use them (122). This so-called "sheath-flow" is provided coaxial to the column and is typically a mixture of methanol, water, and acetic acid (122). The function of the sheath liquid is to dampen any effect of the gradient on the characteristics of the spray (122). As a result, conditions in the ion source can be adjusted or tuned for optimum sensitivity, and that sensitivity is maintained throughout the elution gradient (122).

SDS remnants from the electrophoresis run may also cause problems to the GeLC-MS system (122). They can reduce the sensitivity of the experiment either by competing in some way for the ionization of peptides or by disrupting the spray/evaporation process. SDS in the polyacrylamide gel electrophoresis gives proteins an uniform negative charge which would then neutralize the positive charge given by ESI, making impossible for the peptides to move into the mass analyzer (122). SDS is also a surfactant and thus will affect the surface tension of the solvent droplets containing the peptides inside the ESI device, disrupting the spatial details of the evaporation process established in optimizing the tuning of the ion source (122). The net effect is that the presence of SDS in a sample dramatically reduces the sensitivity of the analysis (122). Fortunately, any trace of SDS or other ionic species that might otherwise interfere with the ionization are removed through the use of the reversed-phase HPLC inlet (122).

1.2.3.2.2 Mass Analyzer

One of the analyzes that is usually coupled to the ESI or Nanospray ionization source used in GeLC-MS/MS experiments is a quadrupole-time-of-flight tandem mass analyser (122). It uses a quadrupole mass filter for the first mass analyzer and an orthogonal time-of-flight mass analyzer for the second mass analyzer (122). The collision cell is a hexapole lens

system that contains and transmits all the ions within the selected *m/z* range (122). For peptide mass measurements, the first quadrupole is used in an rf-only mode to transmit all ions and the time-of-flight mass analyzer carries out the mass analysis (122). The key distinction in a quadrupole-time-of-flight instrument is that mass spectra and product ion spectra are both recorded by the time-of-flight mass analyzer with all of the advantages of time-of-flight analysis previously described in 1.2.2 (122).

1.2.3.2.3 Data Interpretation

The specificity and sensitivity of LC–MS/MS enable simultaneous analysis of multiple components from complex biological mixtures (124). LC is the first dimension of the analysis during which a complex biological extract is chromatographically separated into either individual or overlapping bands of compounds (124). In a LC–MS/ MS method, compounds elute off a LC column into a mass spectrometer which performs as a mass filter (124). The mass filtering provides high selectivity to differentiate among ions formed from different co-eluting analyses (124).

The structural elucidation (amino acid sequence) is performed using data system software to compare the nominal masses of the product ions with the theoretical masses of ions from compounds in a mass spectral library (124), as reviewed in the MALDI- TOF/TOF proteomic analysis (section 1.2.2). Also, in order to identify proteins, bioinformatics tools such as ProteinScape (Bruker) using the MASCOT search engine, identifies proteins on the basis of peptide mass spectra (125). With MASCOT, peptide mass spectra from protein digests are analyzed, assigned a sequence, and protein databases are searched for the presence of the peptide sequences (125).The higher the scores of the peptide sequences identified in the candidate protein, the higher the confidence in the identification (125). The identified proteins can then annotated using gene ontology categories - either manually or using software such as Blast2GO (BioBam) or ProteinScape (Bruker) in order to better understand its biological significance.

1.3 Aims

The objectives of this study are the characterization of the BMP of a highly virulent *S. pseudintermedius* strain isolated from a dog with severe pyoderma (5819/10), previously characterized by MLST as belonging to ST71, the highly spread European-clone (126). Biofilm was developed by culturing the *S. pseudintermedius* strain 5819/10 in specific media. The biofilm matrix was then separated from bacterial cells and evaluated for its protein content and complexity. Finally, the proteome was separated by 1D-electrophoresis and characterized by nanoLC-ESI-Q-TOF and analysed using bioinformatics tools.

2.Materials and Methods

2.1 Workflow

Biofilm from *S. pseudintermedius* strain 5819/10 was grown in TSB enriched with 4% (w/v) of NaCl at 37ºC during 48 hours in a 6-well culture plate using a protocol adapted from the literature (127). Pieces of biofilm matter were taken for light microscopy inspection at several time-points of the growth process. After 48 hours of growth, the biofilm matrix was extracted using a version of a recently published extraction protocol (56), where the amounts of washing buffer and extraction buffer were optimized. The extraction was monitored at several time-points by light microscopy, for which a known staining protocol was first tested and then used (128).

In order to choose the optimal extraction conditions, 5 biofilms grown in wells of a 6-well culture plate, were washed with 330, 660 or 1000 µL of washing buffer and the biofilm matrix was extracted using 100 or 200 µL of extraction buffer- the procedure was monitored by LM. The extracted protein matrix from biofilm samples obtained after different extraction protocols were then run in an SDS-PAGE minigel and stained with Coomassie Brilliant Blue in order to access their profiles and thus select the extraction conditions.

The more intense gel bands were excised, digested with trypsin, and identified using MALDI-TOF/TOF mass spectrometry analysis with the goal of doing a preliminary analisis of the biofilm matrix proteome The protein identification was performed using GPS Explorer™ software (Applied Biosystems) with MASCOT (version 2.2) search engine and the Uniprot/SwissProt database (release 2013_09) restricted to the *Staphylococcus* taxonomy group for protein identification and an initial assessment of the matrix protein extracts was made, to check the enrichment in extracellular proteins.

For GeLC-MS/MS, biofilm from 6 inoculates was grown in petri dishes as well as in wells of 6-well culture plates, aiming to generate higher amounts of protein from biofilm samples, it was necessary to grow the.The quantities of extraction reagents were adjusted for the petri dishes (by a factor ± 6) taking into account the surface area of the vessels. This was essential for complete biofilm extraction.

After extraction, the total protein of all replicates was quantified. The replicates with the biggest protein quantity were selected for GeLC-MS/MS analysis- the biological replicates PD I1 100, PD I3 10 and PD I2 10, as well as the thecnical replicate PD I2 100. About 20 µg of protein from the selected samples were partially run in an SDS-PAGE minigel and stained with Coomassie Brilliant Blue. After that, each lane was cut in 4 bands and the 16 bands were destained, reduced and alkylated and then digested with trypsin.

High Resolution LC-MS/MS analysis was performed for protein identification using Data Analysis 4.2 software (Bruker) to interpret LC-MS/MS and ProteinScape 3.1 (Bruker) using MASCOT search engine (version 2.2) and the Uniprot/SwissProt database (release 2014_05) restricted to the *Staphylococcus* taxonomy group for protein identification. The protein identification data was subjected to protein-protein BLAST (BLASTp) search with BLAST2GO (B2GO) software (BioBam) in order to perform gene onthology (GO) annotation to the identified proteins and better characterize them. A general overview of the GOs from the proteome was performed before proteins related to biofilm formation; cell adhesion, host colonization and pathogenesis were singled out. A comparison between the obtained proteome and already published ones was also undertaken.

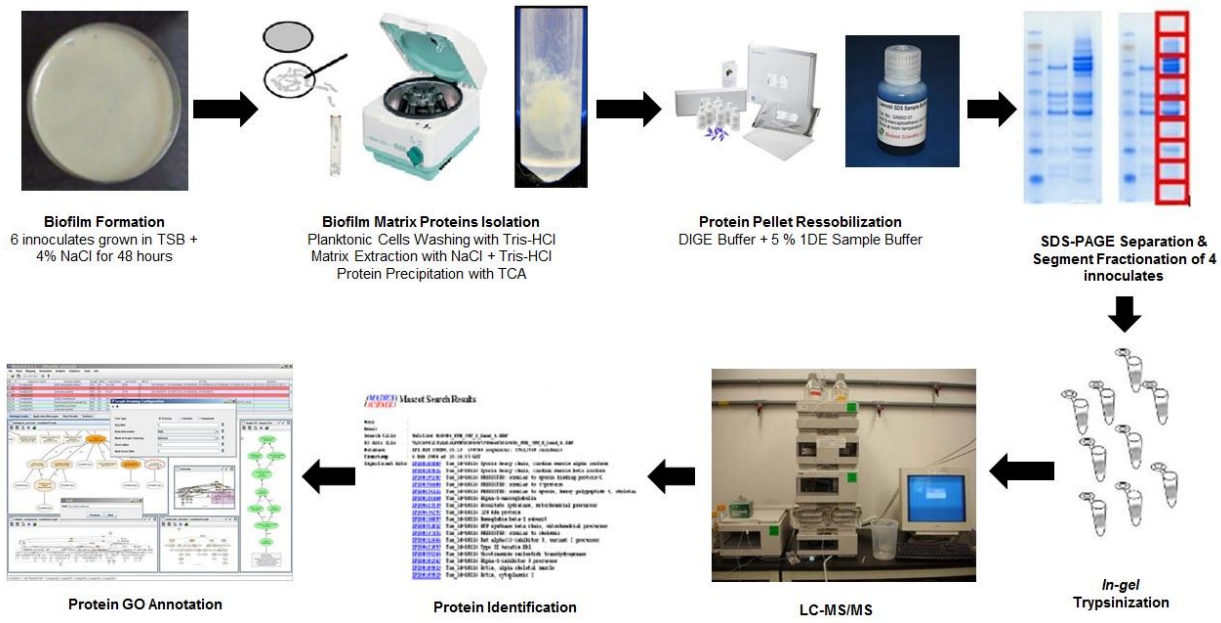

Figure 6- GeLC-MS/MS protocol workflow for protein identification and annotation of S. pseudintermedius 5819/10 biofilm matrix proteome. Biofim matrix protein isolation procedures was optimized by changing the volumes of washing and extraction buffers used and comparing the 1D electrophoresis gel profile of each combination of buffers; a preliminary protein identification of BMP was performed with the optimization protein extracts using MALDI-TOF/TOF. Biofilm formation and biofilm matrix proteins isolation steps were monitored by light microscopy during the optimization procedure

2.2 Detailed Description

2.2.1 Preparation of biofilms

Prior to inoculation and close to a flame, a small amount of stock culture from *S. pseudintermedius* strain 5819/10 was transferred to blood agar and incubated 24 h aerobically at 37ºC. Then, 5819/10 inoculates were prepared by transfer of 3-4 colonies into test tubes containing 5 ml of trypitic soy broth (TSB) – near a flame - and incubated aerobically overnight (ON) at 37ºC. The used strain was first isolated in the Laboratory of Antimicrobial and Biocide Resistance of Faculty of Veterinary Medicine of Lisbon University from a severe case of dog pyoderma (126) and chosen for its virulence (129).The inoculates where then diluted 1:100 the into TSB enriched with 4% (w/v) sodium chloride (NaCl) inside a plastic container, be it the wells of 6 well cell culture plate or 90-mm diameter sterilized dishes, again near a flame. The decision to grow biofilm in TSB enriched with NaCl instead of glucose was made based on previous but still unpublished biofilm growth studies with the straina . Finally, biofilm development occurred throughout 48 hours incubation at 37ºC under static conditions. In the biofilm growth optimization assay, pieces of the growing biofilm were taken after 7, 23, 31 and 48 hours of growth.

For the GeLC-MS/MS assay in particular three inoculates were grown in TSB - as described- and then diluted 1:10 as well as 1:100 as described above. Biofilm from this 6 diferent cultures were grown inside petri dishes and wells in 6-well culture plates. In the end, protein extracts.

2.2.2 Biofilm Matrix Extraction Protocol

This extraction protocol was adapted from a previous protocol (56). First, planktonic cells and the remaining growth medium were decanted and the biofilm matrix was inspected through Light Microscopy (LM), either directly or through a smear on a slide. Then, biofilms of *S. pseudintermedius* formed in the plastic containers were detached by mixing it with a washing buffer (WB) composed of 10 mM Tris-HCl (pH 8.0) and a protease inhibitor cocktail (Sigma-Aldrich). They were then mechanically peeled away from the adherent surface using a cell scrapper and transferred into a test tube.

The yielded biofilm was vortexed and centrifuged at 5,000 x *g* for 10 min. Between the vortex and centrifugation steps, a piece of biofilm was taken for inspection through LM. The supernatant containing reminiscent growth medium and planktonic bacteria was transferred into a new test tube and placed in storage at -80ºC. On the other hand, to the pellet containing the biofilm matrix, a matrix extraction buffer (EB) composed of 10 mM Tris-HCl (pH 8.0), 1 M NaCl, and protease inhibitor cocktail (Sigma-Aldrich) was added. The mixture was then incubated at 25°C for 30 min with gentle rotation. Here, a new inspection by LM was taken. After the incubation, the mixture was centrifuged at 5,000 x *g* for 10 min and the supernatant (the biofilm matrix suspension) was transferred into a new test tube and its contents again inspected using LM. The supernatant comprising the biofilm matrix fraction was then stored at – 20º C, while the pellet containing the removed biofilm bacterial cells was stored at -80ºC.

2.2.2.1 Light Microscopy Protocol

The light microscopy inspection was only performed during the biofilm growth trial the biofilm matrix extraction trial. Biofilm formation was monitored in the former in order to confirm the presence of cell aggregates embedded in matrix of extracellular polymeric substances. During the extraction procedure, light microscopy was used to see the effect that both the washing and extraction buffers had on the biofilm matrix, in terms of removal of planktonic cells in the former and biofilm embedded cells in the latter and to evaluate the removal of planktonic cells during the entire extraction procedure.

After selecting the optimal extraction conditions of biofilms grown in 6-well culture plate wells (as explained in section 3.1.1), buffer volumes were scaled up in order to apply the extraction protocol to biofilms grown on 90 mm petri dishes. The surface area of the culture plate wells and the petri dish were used as references for the scaling calculation, since bacteria adhesion and then biofilm growth is dependent on the surface area of the material (1, 130). The surface area of the petri dishes used was about 6 times the one from a well in 6–well culture plate. Therefore, biofilms grown in petri dishes were washed with 4029 µL of WB and extracted 611 µL of EB.

The staining protocol was adapted from the literature (128, 131, 132). In it, it is stated that biofilm EPS (polysaccharides mostly) generally stains orange/pink with Congo Red solution, whilst Ziehl carbol fuchsin stains bacterial cells purple/red (132).

Biofilm samples were smeared onto glass slides and were covered with 10 mM cetylpyridinium chloride. The slides were allowed to air dry for 20–30 min and then fixed by gentle heating by transient passage over a Bunsen burner flame and allowed to cool. After that, the slides were then stained for 15 min with a 2:1 mixture of saturated Congo Red solution and 10% (v/v) Tween 80, and rinsed in distilled H_2O. Slides were then counter-stained with Ziehl carbol fuchsin 10% (v/v) for 6 min, rinsed in distilled H_2O and dried at 37ºC prior to visualization by light microscopy. After around 30 minutes, the slides were ready to be observed in a LM equipped with the Olympus D-10 digital camera.

2.2.3 Protein Isolation from Biofilm Matrix extracts

Proteins were precipitated using a protocol adapted from the literature (101). Proteins were isolated through precipitation, using 20% (w/v) trichloroacetic acid at 4ºC until achieving a final concentration of 10%. Following 30 min of incubation, the samples were centrifuged 16,000 x *g* for 30 min at 4ºC. The supernatant was discarded and the precipitates were washed twice with cold acetone and the pellet recovered after a centrifugation step at 16,000 x *g* at 4ºC. The pellets were dried at room temperature and stored at -20ºC until further use.

2.2.4 Protein quantitation

Proteins pellets were first ressolubilized in the DIGE buffer (urea 7M, thiourea 2M, Triton X-100 2 % (v/v), Asb-14 0.5 % (m/m), DTT 50mM). Then they were quantified using the 2D QuantKit (GE Healthcare).

2.2.5 SDS-PAGE coupled with MALDI-TOF/TOF Mass Spectrometry for preliminary protein extracts evaluation

2.2.5.1 SDS-PAGE

Proteins were concentrated in a 5% (w/v) acrylamide 7-cm stacking gel and separated in a 12.5% (w/v) acrylamide resolving gel. To the protein samples previously solubilized with solubization buffer, 5% of 1DE sample buffer (62.5 mM Tris-HCl pH 6.8, 20% glycerol, 2% SDS, 5% β-mercaptoethanol e bromophenol blue powder) was added. The electrophoretic separation was performed at 50V for 15 min and then 150V for 1 h. PageRuler Unstained Protein Ladder (Thermo Scientific) molecular mass standard was run along with the samples. After the run, the gel was stained with Coomassie Brilliant Blue and photographed.

2.2.5.2 Protein reduction and alkylation

Gel bands of interest were excised and transferred to different tubes. Then 100 µL of acetonitrile (ACN) was added to each tube and incubated for 15 min. The ACN was removed, 50 µL of 10mM dithiothreitol (DTT) in 100 mM NH_4HCO_3 was added, followed by 45 min incubation at 56ºC, in order to reduce disulphide bridges. Next, the liquid was discarded and 50 µL of 55 mM iodoacetamide (IAA) in 100 mM NH_4HCO_3 was added. This was followed by 30 min incubation in the dark at room temperature in order to alkylate the previously reduced dissulphide bridges.

2.2.5.3 *In gel* digestion of proteins

The gel bands were dehydrated with 100 µL ACN for 15 min. This step was repeated until the gel bands were white and then the bands dried in a sample vacuum concentration system (SpeedVac) for about 10 min. The gel bands were rehydrated in a digestion buffer (6.7 µg/ml trypsin in 50 mM NH_4HCO_3) and incubated for 15 min at 4ºC. More digestion buffer was added when needed to completely cover the gel bands. Following an incubation period of 45 min at 4ºC, the remaining buffer was removed and 50 mM NH_4HCO_3 was added to cover the gel bands. The digestion step took 14h at 37ºC. Formic acid was added, up to a final concentration of 5%, in order to stop the reaction. Finally, after an incubation period of

15 min under agitation, the supernatants containing the peptide digests were transferred to new tubes and both bands and digested peptides were stored at -20ºC until further use.

2.2.5.4 MALDI-TOF/TOF

2.2.5.4.1 Sample preparation

The tryptic peptides were acidified with 5% (v/v) formic acid, concentrated with POROS R2 microcolumns (GELoader tip, Eppendorf) and co-crystallised in MALDI-TOF/TOF sample plates using the matrix α-cyano-4-hydroxycinnamic acid (Sigma).

2.2.5.4.2 MALDI-TOF/TOF data acquisition and protein identification

Tandem mass spectrometry was performed using a MALDI-TOF/TOF 4800 plus mass spectrometer (Applied Biosystems). The mass spectrometer was externally calibrated using des-Arg-Bradykinin (904.468 Da), angiotensin 1 (1296.685 Da), Glu-Fibrinopeptide B (1570.677 Da), ACTH (1-17) (2093.087 Da), and ACTH (18-39) (2465.199) (4700 Calibration Mix, Applied Biosystems). Each reflector MS spectrum was collected in a result-independent acquisition mode, typically using 1000 laser shots per spectra and a fixed laser intensity of 3500V. The fifteen strongest precursors were selected for MS/MS, the weakest precursors being fragmented first. MS/MS analyses were performed using CID (Collision Induced Dissociation) assisted with air, with collision energy of 1 kV and gas pressure of 1 x 10^{-6} torr. Two thousand laser shots were collected for each MS/MS spectrum using a fixed laser intensity of 4500V.

Proteins were identified using GPS Explorer™ software (Applied Biosystems) with MASCOT (version 2.2) search engine and the Uniprot/SwissProt database (release 2013_09) restricted to the *Staphylococcus* taxonomy group. Searches were performed in the MS/MS ion search mode and the parameters were set as follows: minimum mass accuracy of 30 ppm for the parent ions, an error of 0.3 Da for the fragments, one missed cleavage in peptide masses, and Cys carbamidomethylation and Met oxidation as fixed and variable amino acid modifications, respectively. Peptides were only considered if the ion score indicated extensive homology ($p<0.05$).

2.2.6 GeLC-MS/MS for proteome characterization

2.2.6.1 Gel Electrophoresis

Four proteins was subjected to partial 1D-electrophoresis and protein digestion steps as in sections 2.2.6.1-3 but with some differences. The SDS-PAGE run was stopped at 2/3s of the run and each lane was cut in 4 bands. In-gel digestion trypsin digestion was performed at a 5:1 ratio of protein: trypsin (w/w). After digestion, the supernatants were transferred to new tubes and then lyophilized and stored at -20^{o}C in preparation to the LC-MS/MS analysis.

2.2.6.2 LC-MS/MS

The samples were analyzed on a Maxis Impact Q-TOF spectrometer (Bruker, Bremen), coupled to a nano-HPLC system (Proxeon, Denmark). The samples, dissolved in 5% acetonitrile, 0.1% formic acid in water, were first concentrated on a 100 µm ID, 2cm Proxeon nanotrapping column and then loaded onto a 75 µm ID, 25 cm Acclaim PepMap nanoseparation column (Thermo). The chromatography was runned using a 0.1% formic acid - acetonitrile gradient (2-30% in 120 min for total lysates digests at a flow rate 300 nL/min). The column was coupled to the mass spectrometer inlet through a Captive Spray (Bruker) ionization source. MS acquisition was set to cycles of MS (2Hz), followed by 3 second cycles of MS/MS (4-16Hz, intensity depending) of a variable number of the most intense precursor ions, with an intensity threshold for fragmentation of 2000 counts, and using a dynamic exclusion time of 2 min, with an automated precursor re-selection when a 3 fold increase in intensity was observed. All spectra were acquired on the range 150-2200 Da. LC-MS/MS data was analyzed using the Data Analysis 4.2 software (Bruker).

Proteins were identified using the Data Analysis 4.2 software (Bruker) to interpret LC-MS/MS and ProteinScape 3.1 (Bruker) using MASCOT search engine (version 2.2) and the Uniprot/SwissProt database (release 2014_05) restricted to the *Staphyloccocus* taxonomy group. MS/MS spectra were searched with a precursor mass tolerance of 10 ppm, fragment tolerance of 0.05 Da, trypsin specificity with a maximum of 2 missed cleavages, cysteine carbamidomethylation set as fixed modification and methionine oxidation as

variable modification. Significance threshold for the identifications was set to $p<0.05$, minimum MASCOT ions score of 20.

2.2.6.3 Protein identification criteria, BLASTp searches and gene ontology annotation

Protein identification reports were generated containing the information of all the matched peptides and proteins. Protein identifications were considered if the protein score indicates significant statistical confidence ($p<0.05$). Protein identifications with only one peptide with 95% confidence were done using two additional quality criteria: sequence coverage of $\geq 10\%$ and a deviation of predicted mass, RMS90 of ≤ 50 ppm.

A BLASTp search was performed through BLAST2GO (BioBam) java application (http://www.blast2go.de). This enabled to perform GO annotation of the identified proteins by using GO categories of the best hit derived from the BLASTp results (BLASTp minimal Expectation value set to $< 1x10^{-3}$).

Non-caractherized proteins were excluded from further data treatment while the rest were characterized by their cellular component, molecular function and biological process GO terms. The annotated proteins were also compared to known biofilm cells and matrix homologous proteins from bacterial strains of *E. coli*, *Pseudomonas auruginosa* and *S. aureus* in order to highlight biofilm matrix related proteins and other virulent proteins. Known proteins associated to biofilm formation, cell adhesion, host colonization and pathogenesis were singled out.

3.Results and Discussion

3.1 Biofilm Formation and Growth

Biofilm formation and growth was monitored using light microscopy (figures 7-12). Samples of a biofilm grown in one plate of a 6-well culture plate were taken after 7, 23, 31 and 48 hours after which they were fixed and stained using a protocol previously described (128, 131) but not previously used in *Staphylococcus spp*. The figures show that it provided enough contrast between cells, biofilm matrix nearly stripped of bacteria and denser biofilm matrix full of bacteria.

After 7 hours of growth (fig. 7), it was possible to observe the formation of globular-like aggregates of EPS of diferent size and color in what appears to be different stages of development (fig. 7-A). Some of them even have an interior darker than others, which are only formed by a brighter matrix substances. The bright tissue might be the beginning of what is called the loosely-bound EPS (LS-EPS), which is a type of biofilm matrix gluing clusters to form microcolonies and flocs (133). The dark tissue on the other hand might be the tightly-bound EPS (TB-EPS), which is found on the cell wall, which links cells together in clusters, and are generally cell wall associated proteins (133). Also observed, are membrane-like tissues composed of the darker material surrounding a sea of the lighter material with embedded bacteria, which fits the description of a biofilm matrix (fig 7-B).

The growing biofilm does not appear to be formed homogeneously. Not only matrix-like structures and globules so thick that resemble a fully-formed biofilm (figure 7-A) are observed, bacteria surrounded by a thin coating of EPS (in fig 7-C) and circular structures interconnected by a matrix (fig. 7-D) are also present after 7 hours of growth. The EPS connected circular structures may point towards a biofilm that is first formed in small patches and then form the connecting tissue between themselves and finally, to close the net they produce a polymer rich structure so that appears to be a differentiated type of biofilm matrix.

On the other hand, the differences observed could be related to biofilm formation through different means: PIA-dependent or -independent and eDNA-dependent biofilm formation. However, the data presented cannot prove this hypothesis and no proteomic analyses was performed at this stage. The clearest evidence these micrographs provide is that first, the staining protocol provides the distinction between cells and different types of EPS and second, that biofilm growth and maturation is not uniform.

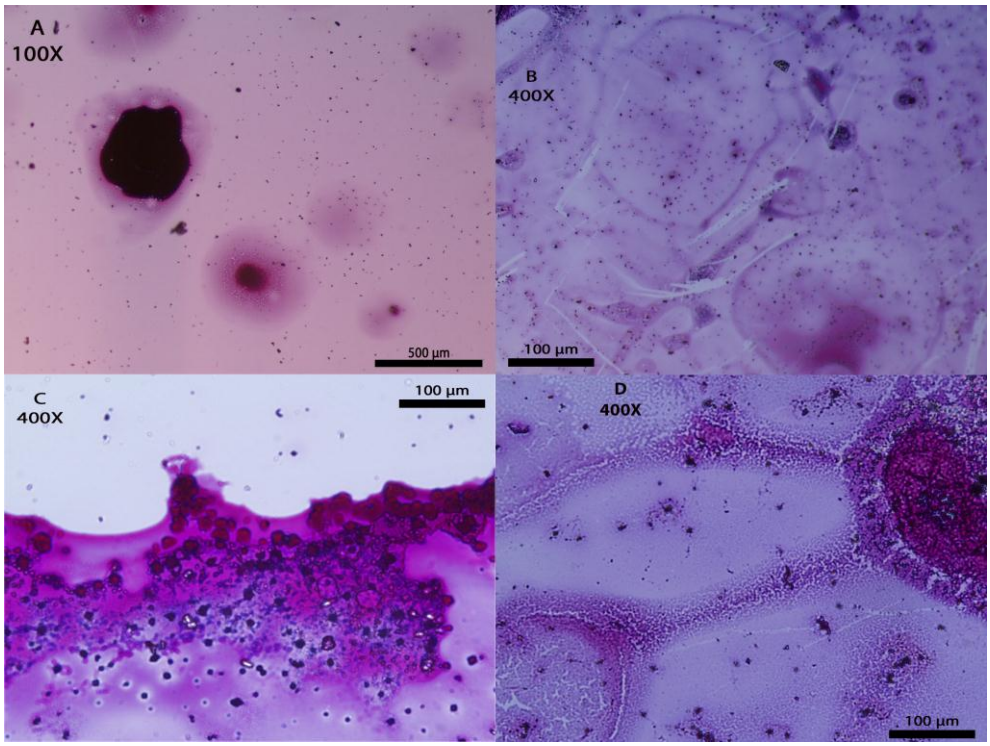

Figure 7- Different stages of biofilm formation after 7 hours of growth. In A there's a circular structures composed of dark matrix surrounded by light matrix. In B, small cocci are embedded in semi-transparent light purple matrix. C shows a section of darker but still light purple matrix that separates the interior of the matrix from the exterior and is full of embedded cocci. D, on the other hand, shows the interior of a section of matrix where large round structures of slightly darker purple matrix are interconnected by bridges in the same color and a thinner matrix.

After 23 hours the biofilm seems generally denser, though different stages of development are still observed (fig. 8). Notably it became apparent the development of canals throughout the denser structures (fig 8-C and D) and the opening of pockets in both types of EPS (fig 8-B and D).

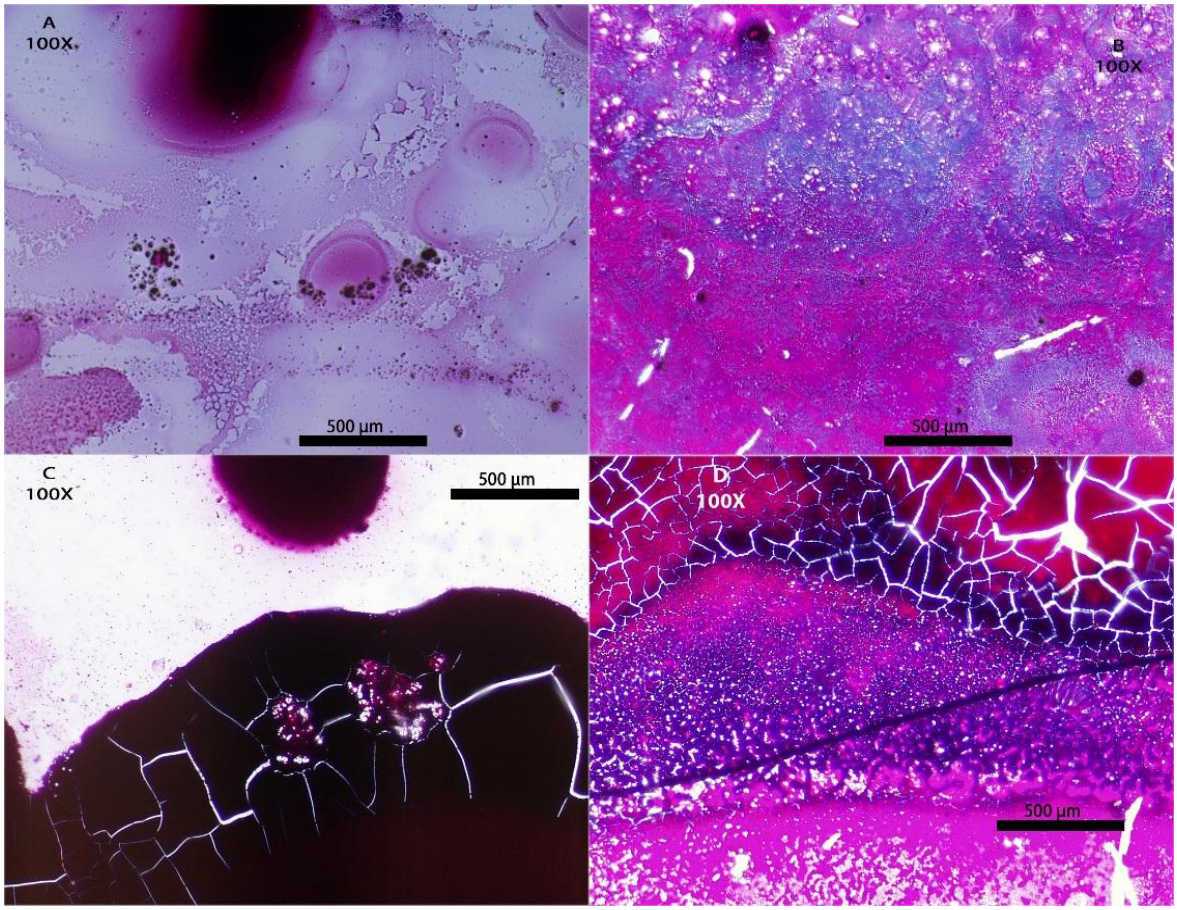

Figure 8- Stages of biofilm formation after 23 hours of growth. A shows a portion of dense biofilm matrix surrounded by thinner matrix. B highlights the thickening of lighter-colored biofilm matrix as the biofilms developed while C shows that denser biofilm matrix had already developed as well as internal channel. D shows a frontier region between region between different types of EPS where the thinner matrix merges with the denser one. The thinner part of the matrix seems to become darker and thicker as it gets closer to the denser part possibly indicating an area of intermediate matrix density.

The darker matrix seems to be a very differentiated and dense structure (figs 9-C and and 9-A, respectively). Congo red stains polysaccharides reddish and carbol fuchsin, the bacteria blue/pink (128, 131). Both of these hues are prevalent here, but some globules, canals, and pockets are clearly visible still (figs 9-B and 9-D). There also seems to be bacteria traveling through the channels in fig 8-A, though one has to keep in mind that LM provides 2D images and does not guarantee that both channels and bacteria are in the same plane or just superimposed.

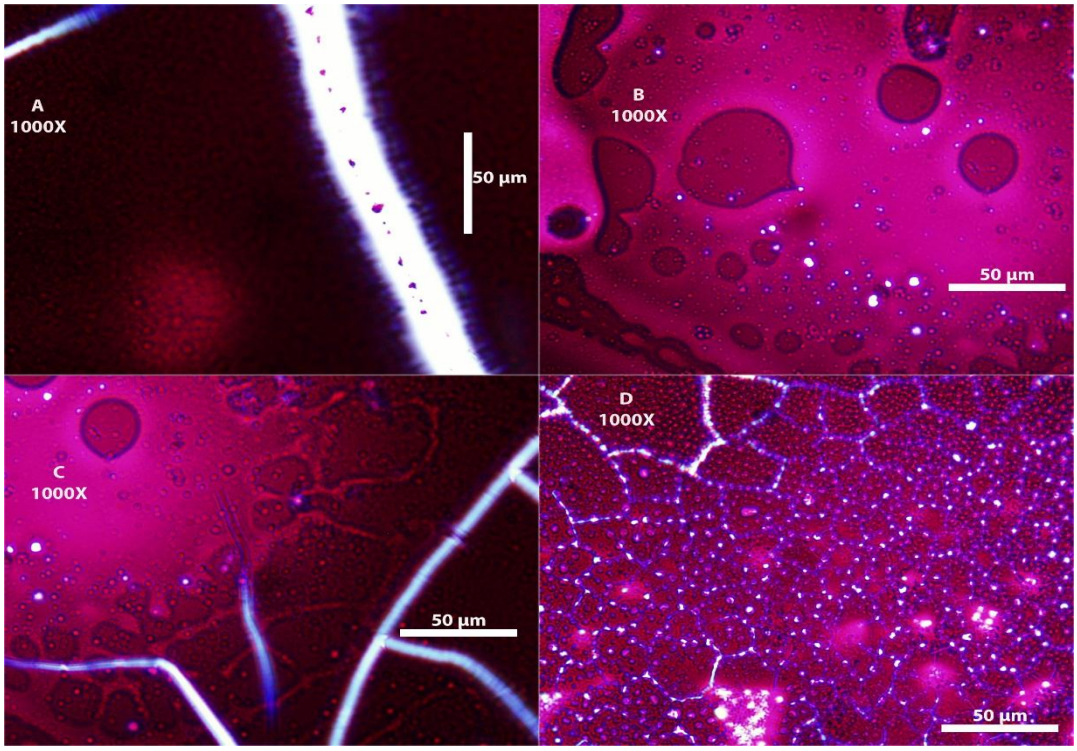

Figure 9- Closer look at darker biofilm matrix formed after 23 hours of growth. A highlights a channel within a very dark purple portion of matrix with what appears to be cocci aggregates traveling through it. B shows a slightly pinkish matrix containing some pockets and darker globules while C shows a different part of same section of the matrix where channels coming from said part of the matrix. D highlights the existence of channels and pockets throughout this denser part of the matrix.

After 31 hours of growth, there was evidence of a more mature and differentiated biofilm (Fig 10-B), but also one very fragmented (fig 10-A,10-D 11-B and C for ex.). It was possible to observe bacteria glued by a purple matrix (fig 11-B and C). In other micrographs (fig 10-A and D) a lack of a lot matrix is observed as if the biofilm was ripped apart. It was not expected, but it might just show that the biofilm matrix sample observed in these pictures did not possess a highly mature biofilm or simply the biofilm was not sufficiently fixed during the staining procedure and washed away when rinsing the slides between changing dyes.

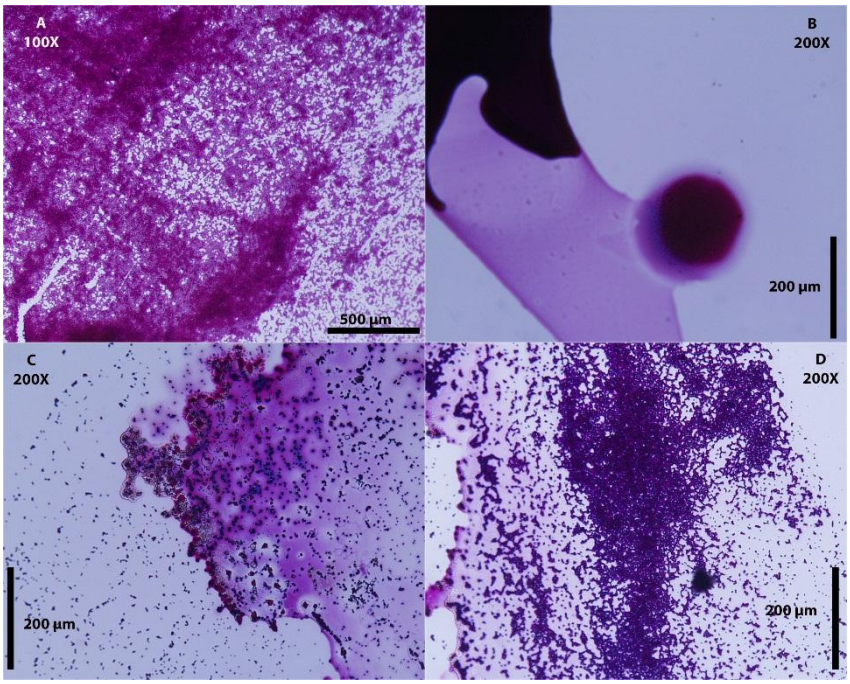

Figure 10- Biofilm after 31 hours of growth. A very fragment biofilm with small pieces close to a very fragment bulk is observed (A). In stark contrast, an integral matrix where dense section of matrix are interconnected by a large thinner section (B) is also present. Around the globule in B there's also a hallow of intermediate density matrix similar to the matrix in fig.7-D. C shows a piece of thinner matrix embedding a small amount of bacteria while D shows mostly cocci aggregates neighboring a near existent matrix.

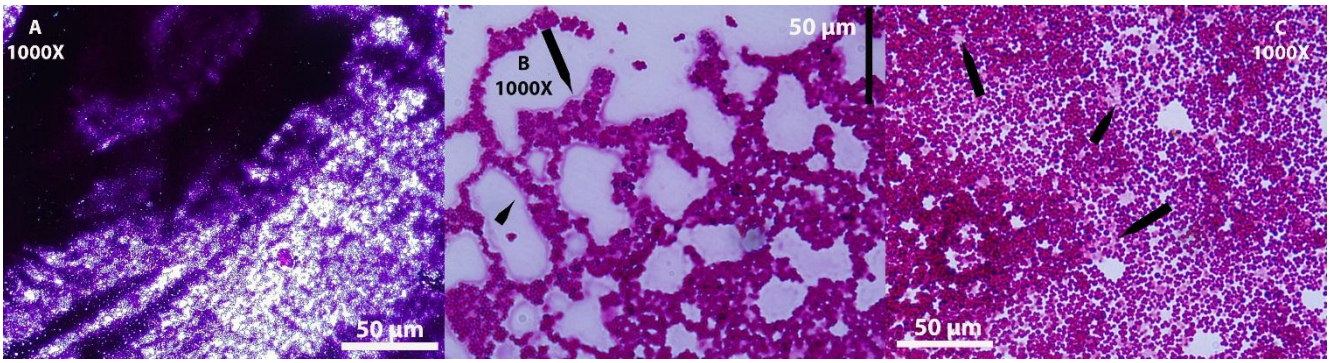

Figure 11- Closer look 31 hours old biofilm. A shows an evolution of the previous stage while B and C highlights a less darker and presumably mature biofilm. The arrows in B and C point towards the matrix that surrounds and supports the bacteria.

In the last stage of the growth period (fig 12), the biofilm matrix looked like a mixture of the one pictured after 27 and 31 hours (figures 8-11), but with some differences. There was a bluish biofilm matrix (fig 12-A, B and C) as well as a thick purplish biofilm matrix full of channels (fig 12-E). This highlighted the existence of both polysaccharide rich matrix and bacteria rich biofilm matrix. Figures 12-B and C shows large aggregates of cocci on a

matrix support and in figures 11-A and C it was possible to see blue cocci aggregates and light purple matrix with or without bacteria directly connected to a dark purple biofilm containing a large amount of bacteria embedded in EPS. This evidence were in agreement with scanning electron microscopy studies on *S. pseudintermedius* biofilms that have shown that the biofilm matrix consists in bacteria embedded in EPS with large aggregates of cocci on the surface (34).

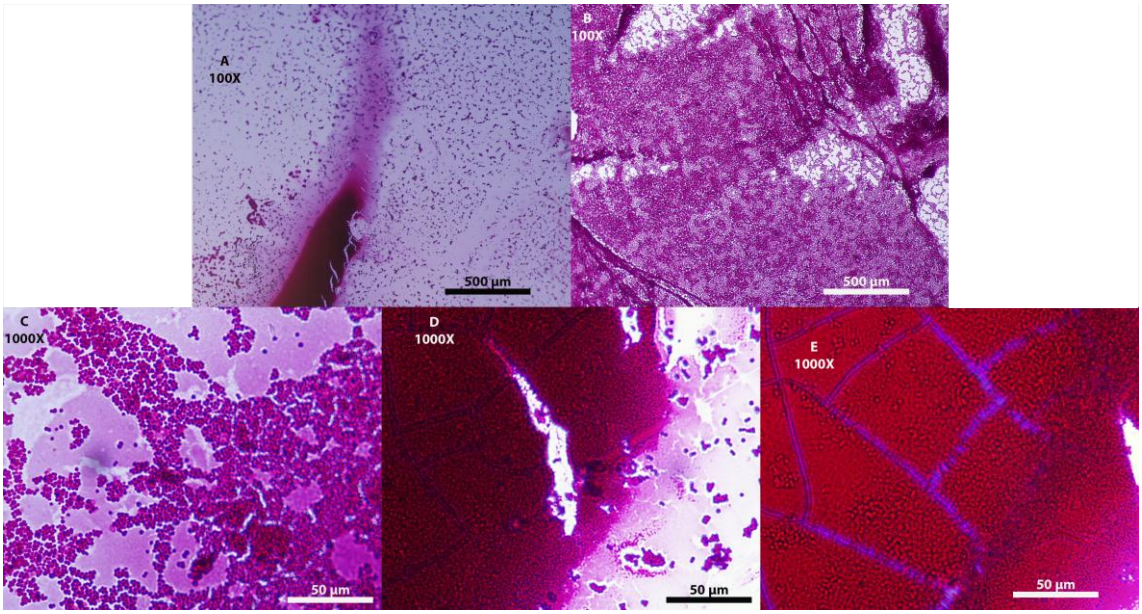

Figure 12- Biofilm before extraction (after 48 hours of growth). Different types of matrix are present. A shows a matrix consisting of denser matrix attached to thinner one in which some cocci are embedded. In B, embedded in light purple matrix there's a large amount of cocci which are further highlighted in C. D is a close up. D shows in more detail an area of the same matrix depicted in A where the dark matrix merges with the lighter one and where cocci aggregates are present. E shows a purplish matrix full of channels and bacteria.

3.1.1 Biofilm extraction: protocol development and optimization

The monitoring of the extraction procedure was done immediately following the biofilm formation and growth experiment, using the remaining 5 biofilms cultivated alongside the one sampled for the growth monitoring. Five different conditions (table 2) were experimented based on the data provided by the literature protocol: three different volumes of washing buffer (WB) were tested and coupled with two different volumes of extraction buffer (EB).

Table 2- Volumes of washing and extraction buffer used for protocol development

Sample Code	Volume (µL)	
	Washing buffer (10 mM Tris-HCl (pH 8.0) & protease inhibitor cocktail)	Extraction buffer (10 mM Tris-HCl (pH 8.0), 1 M NaCl & protease inhibitor cocktail)
E32	330	200
E61	660	100
E11	1000	100
E12	1000	200

In order to find the most appropriate extraction procedure, the extraction was monitored by light microscopy and by 1DE coupled with MALDI-TOF/TOF. The protein extracts were solubilized in 1DE sample buffer and 20 µL of dissolved samples were loaded into a SDS-PAGE mini-gel. Along with most samples from the biofilm matrix extraction assay, a sample obtained using another extraction run using j the most extreme version of the extraction protocol (the same as sample E12)) was loaded into the gel (sample TBM 2nd Exp).

On the gel profile is shown in figure 13, nine bands with similar profiles were detected on most of the lanes. This indicates that the different extraction procedures did not induce differences on the bulk of the protein extracted. Differences in protein band density were observed between lanes and they do not follow a pattern that clearly established a proportion between band densities with the amount of extraction buffer added. Most glaring, the bands in sample E12 were denser than E11, but this sample has bands more intense than TBM 2º E, which is a sample extracted the same way as E12. The differences in band density were mostly attributed to the different amount of protein in the loaded samples, since at this stage the samples were not quantified. Yet, the extraction procedure using 660 µL of WB and 100 µL of EB, provided the gel profile with the denser bands. Attending that the same volume of protein extract was loaded in each well, it can be assumed that sample E61 had the higher protein concentration. Having also provided a similar gel profile as the rest, this

extraction procedure was then selected for the remaining extractions and basis for the biofilm production scaling-up by 6 fold from the 6-well culture plate to the 90-mm petri dishes.

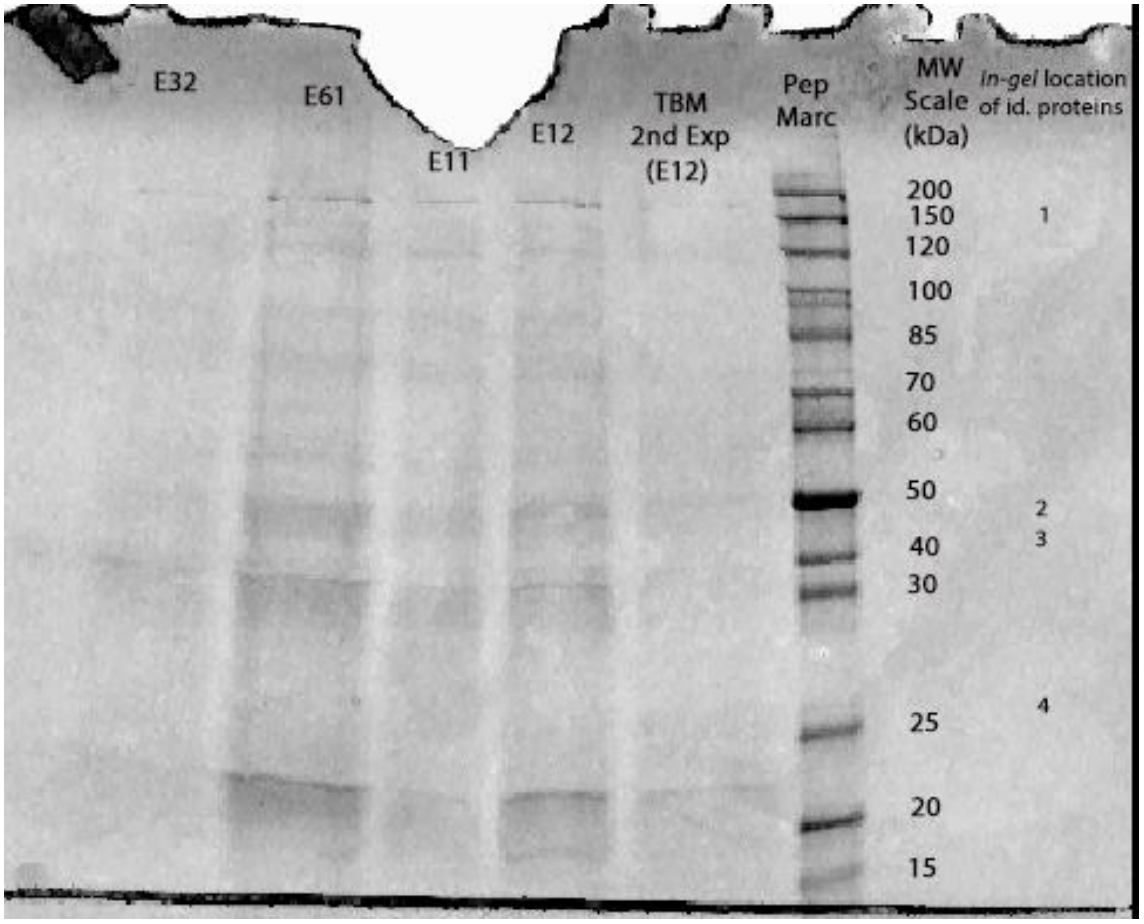

Figure 13- 12% polyacrylamide SDS PAGE gel profile of some of the samples extracted. Samples are the same as described in the table 2 with the addition of the TBM 2º Exp that is similar to the E12 sample, but was extracted separately The protein marker is the PageRuler Unstained Protein Ladder from Thermo Scientific. 4 proteins were identified and their position along the gel is shown based on their MW- more information is presented on table 3 in the next section.

The extraction procedure was monitored by light microscopy at 3 time points (fig 14):

1. After the addition of the washing buffer followed by quick vortexing;
2. After the addition of extraction buffer followed by 30 minutes of incubation under mild agitation;
3. After the extraction: sampling of the resulting suspension.

The addition of washing buffer (figs. 14-1A and 14-1B) removed the majority of planktonic coccus when compared to pre-extraction biofilm shown in figures 13A. There was still big and opaque/dense dark purple circular structures and inside the "membranes" of the biofilm, a wide open space. This showed that the washing buffer actually removed a large amount of biofilm matter by itself. However looks can be deceiving because in later extraction stages (figs 14-2B and 14-3A), they were present as well as in every test condition monitored (including the presented one but in too blurred images). Interesting, the hollow space of figures 14-1A and 14-1B was filled with filaments of biofilm matter that demark what appeared to be sheets of biofilm matter and some coccus aggregates. The retention of this material could be evidence that the biofilm was not homogenous and that some parts were denser or more resistant than others.

The addition of the extraction buffer did not change the appearance of the biofilm too much. Comparing figures 14-1A and 14-1B with 14-2A, there was still an outer biofilm membrane with a dense aggregate and coccus. The major difference was the larger amount of coccus in the latter image. This could be either the result of a release of bacteria from large cocci aggregates inside the matrix or from denser biofilm material. There was also a smoothing of the biofilm "membranes" which could offer support to the latter hypothesis.

Neither washing nor extraction buffers destroyed the complex biofilm structure with all its different densities in biofilm matrix and channels within as well as cocci aggregates (figure 14-2B). However, after centrifugation the state of the biofilm became different. At the end of the extraction procedure, there was still both denser and lighter types of biofilm matrix (together or not) and a few cocci (figs. 14-3A and 14-3B). Figure 14-3A shows a biofilm with more holes, less denser material and no visible cocci compared to figure 14-2B. On the other hand, figure 14-3B shows a biofilm similar to 14-2A but much hollower and fragmented, though still some cocci are observed. It seems as if the extraction buffer loosened the biofilm matrix and centrifugation removed the loosened material. Supporting this hypothesis, a *S. aureus* biofilm subjected to a similar treatment lost its surface-adherent materials and the internal structure of the biofilm was exposed (56).

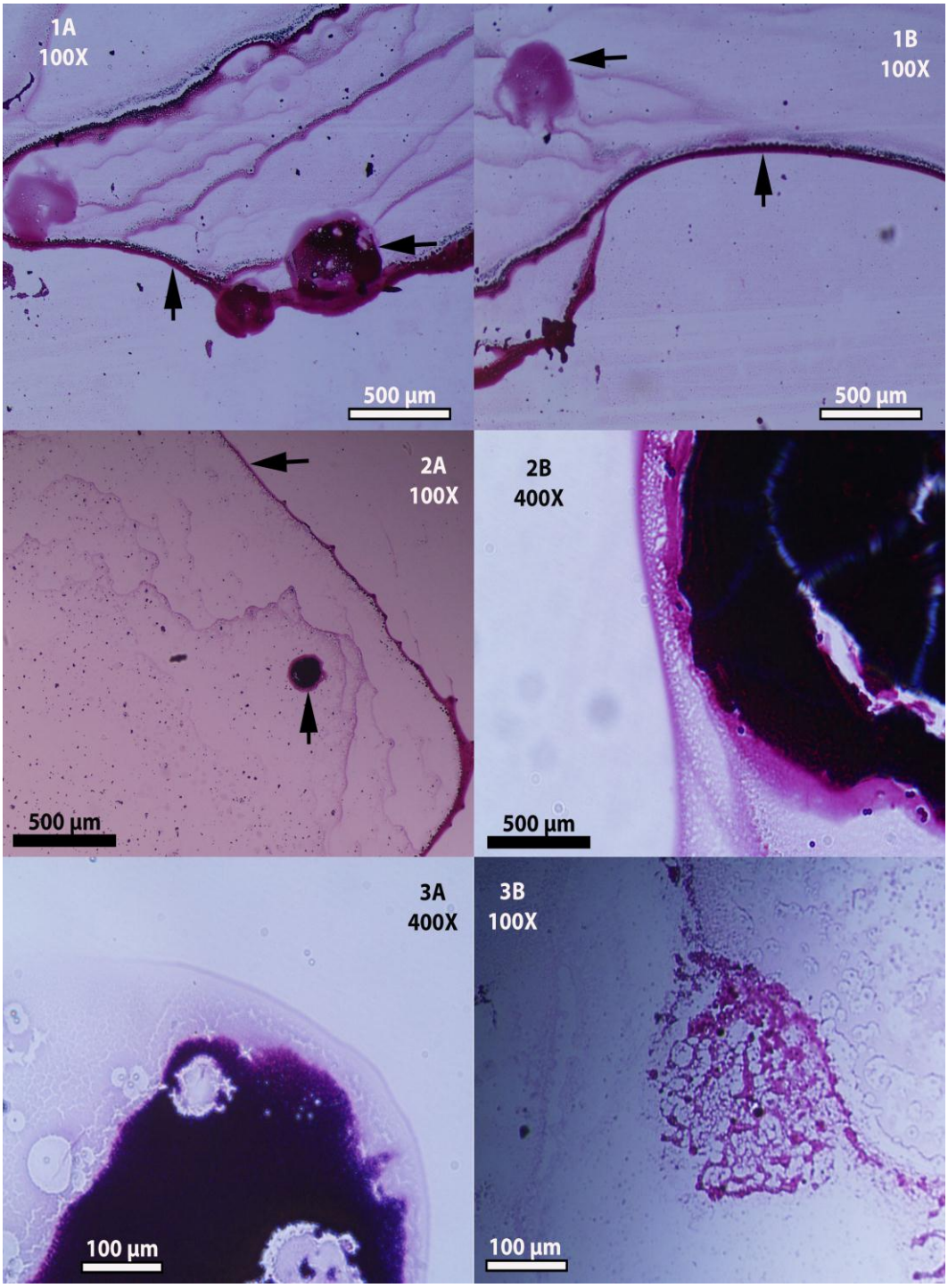

Figure 14- Light microscopy monitoring of the extraction procedures. Images 1A and 1B shows the state of the biofilm after addition of washing buffer. The matrix is mainly clean of cocci aggregates both inside and outside the matrix, while denser parts of matrix are visible. 2A and 2B shows the state of the matrix after the addition of the extraction buffer. In 2A a cleaner version of 1A and 1B is present where the border the matrix and its inner structures are thinner while a dark globule was not removed. 2B confirms shows that denser sections of biofilm matrix still remain after washing and addition of extraction buffer. Persistent. However, after been shaken and centrifuged, the matrix was ripped apart with large holes forming in denser matrix (3A) as well as in the lighter matrix (3B). In the latter, a darker section of matrix similar to a peninsula looks like a globule similar to the one in 1A that was shredded to pieces.

3.1.2 Protein Identification

To evaluate the optimized matrix and protein extraction protocols, the more intense 1DE gel bands of the total protein extracts were submitted for protein identification using MALDI-TOF/TOF data. The more intense gel bands from samples E61 and E12 were excised, digested with trypsin and analyzed by MALDI-TOF/TOF. The combined (MS+MS/MS) data obtained were then matched with the Uniprot/SwissProt database (release 2013_09) restricted to the Staphylococcus taxonomy group the *S. pseudintermedius* protein database through MASCOT (version 2.2) on the GPS Explorer™ (Applied Biosystems) software.

Four staphylococcal proteins were identified with 3 of them being extracellular ones. The main evidences obtained from this preliminary study are that most proteins have a rather small molecular mass and are similar to those found in both *S. aureus* and *S. epidermidis* studies, notably Atl and Eap. The former is usually a CW-protein, but it can be found on the biofilm matrix. As for the latter, it obtained the biggest protein score here and was also one of the most expressed proteins in a *S. aureus* study in which a similar extraction protocol was used.

The preliminary protein identification assays by MALDI-TOF/TOF were used to check if the main protein bands belonged to biofilm matrix proteins. For the proteome characterization the proteomic procedure was changed to GeLC-MS/MS. and due to the high complexity of protein extracts and to remove any contaminant that co-precipated with the proteic matter when the biofilm matrix extracts were previously subjected to trichloroacetic acid precipitation. This method allowed an initial fractionation of the protein extracts and the removal of impurities in the first-dimension PAGE run, while high resolution liquid chromatography provided a powerful separation and high confidence in protein identification.

Table 3- Proteomic Data retrieved from the MALDI-TOF/TOF analysis followed by comparison with Uniprot/SwissProt database (release 2013_09) restricted to the bacteria taxonomy group

Protein Name	Accession Number|Entry Name	Gene	Location in figure 12 (number)	Protein MW (kDa)	Protein Score	Total Ion Score	Pep. Count	Intensity Matched	Location	Relevant Functions
Bifunctional autolysin Atl	E8SEU0|E8SEU0_STAPH	*SPSINT_0746*	1	150.67	107	107	2	7,019	cell wall protein involved in peptidoglycan catabolic process	N-acetylmuramoyl-L-alanine amidase activity
Elongation factor Tu	E8SJ27|E8SJ27_STAPH	*tuf*	3	43.19	232	221	4	11,011	cytoplasm	Promotion of the GTP-dependent binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis
Enolase	E8SKF4|E8SKF4_STAPH	*eno*	2	47.00	432	404	7	15,156	Cytoplasm, Cell surface, and cell exterior (is a secreted protein)	Catalysis of reversible conversion of 2-phosphoglycerate into phosphoenolpyruvate. Essential for the degradation of carbohydrates via glycolysis
Extracellular adherence protein of broad specificity Eap/Map	E8SIY6|E8SIY6_STAPH	*SPSINT_1487*	4	27.42	1050	966	17	38,964	Extracellular region	Pathogenesis

3.2 Proteomic Analysis by GeLC-MS/MS

3.2.1 Protein Quantitation

For this analysis, biofilms of six inoculates (biological replicates) were grown in petri dishes as well as in a 6-plate culture plate. The buffers' quantities used in the scale-up extraction protocol in the petri dishes were proportional to the surface area (for bacteria adhesion) of plates of culture plate (about 6 times more). All the samples were quantified

using 2D-Quantkit followed by a 1D SDS-PAGE of 5 µg of total protein to visually confirm the quantification and estimate a concentration to use in the proteomic assay (5.1 Annex).

Four biological replicates with similar gel profiles and a protein concentration (around 2 µg/µL) were selected for the proteomic assay. About 20 µg of each were loaded into a SDS-PAGE gel, and the electrophoresis was interrupted when the fringe beacon reached 2/3 of the cassette. After that, the gels were stained with Coomassie Blue and then destained, the gel profile was analyzed and it was decided to cut each lane into four bands and digest them with trypsin. The supernatant was removed, dried and stored at -20ºC until performing the LC-MS/MS assays.

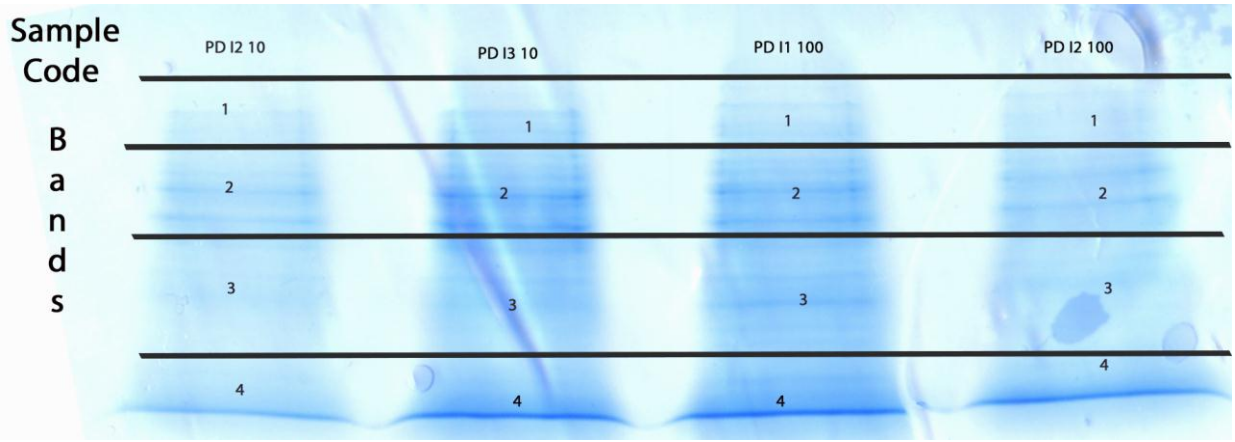

Figure 15- Schematics of the way gel bands were cut in minigel used in the first stage of GeLC-MS/MS. The dark lines indicate the cutting positions of the several gel bands used for tryptic digestion before the GeLC-MS/MS assays.The sample codes refers to the actual biofilm samples. PD refers to the plastic 90 mm petri dishes, the support material where the biofilms were grown. The "Is" refers to the different inoculates and the "10s and 100s" are references to the dilution factors of the inoculates prior to biofilm grown: 1:10 and 1:100 respectively.

3.2.2 Biofilm Matrix Proteome

3.2.2.1 Gene Ontology Overview

A total of 746 unique proteins were identified from the LC-MS/MS analysis (Section 5.2, Annex) when the results from all replicates were combined. Proteins where then mapped and annotated with Blast2GO (BioBam). Of these, 655 proteins had GO terms assigned to them, with 584 being directly identified proteins from the database, while 71 were identified from homology. The identified and annotated proteins were then compared with BMPs from *E. coli* MG1655, *P. aeruginosa* and *S. aureus* MR23 (56, 95, 134). Additionally the

published cellular proteomes of *S. aureus* COL and N315 were also included in the comparison (135). From the 655 identified and annotated proteins, 126 homologous proteins were found in the published strains (table 5), with 24 proteins being previously described in the biofilm cells *of S. aureus* COL and N315. Percentage wise, most proteins had homologs in the *E. coli* matrix proteome while in absolute terms, with the three *S. aureus* strains matrix and cellular proteomes.

Several *S. pseudintermedius* proteins had homologs with *P. aeruginosa* PAO1 but percentage wise they were relatively few. These differences can be explained when one takes into account that most of *P. aeruginosa* biofilm matrix proteins were found on outer membrane vesicles present in the biofilm matrix (134). The proteins found within them were significantly different from the ones of *P. aeruginosa* planktonic outer membrane vesicles (134), which points to specialization of the *P. aeruginosa* biofilm. However, these structures are yet to be described in the *S. pseudintermedius* biofilms and are not likely to, since they are only produced by Gram-negative bacteria (136).

Table 4- Numerical summary of LC-MS-MS analysis

Type of identification	nº of proteins
Directly identified Proteins with GOs	584
Blasted Proteins with GOs	71
Total identified and annotated Proteins	655
Total Proteins without GOs	91
Total Proteins	746

Table 5- Comparion between the number of proteins found o S. pseudintermedius 58910 and other bacterial strains

Bacteria	Nº of identified proteins	Nº of homologous proteins	% homologous proteins
P. aeruginosa PAO1	178	26	14,6
E.coli MG1655	40	32	80,0
S. aureus (COL, MR23 & N315)	130	68	52,3

Blast2Go (BioBam) allowed the attribution of cellular components, molecular and biological functions to the identified proteins using their GOs. A threshold of 10 protein sequences allocated to the same GO term were selected for further analysis. (about 1,5 % of proteins with GOs coverage). Also, it is important to know that not every annotated protein has a GO for each class but some have more than one GO for each class. This meant that the number of GOs in each graphic do not correspond to the number of annotated proteins (655).

Following the established criteria and regarding the cellular component attributed to each protein sequence, the data shows that 242 assignments were done, of which 63% could be related to either the extracellular region or the plasma membrane, such as protein complexes (fig. 16). On the other hand, cell-wall associated proteins did not make the selected sequence coverage cut-off and so, do not appear in the GO pie-chart (fig 16). Also, a relevant number of proteins were attributed to ribosomes and a few to cytosol, which was also observed in the BMPs of *E. coli*, *P. aeruginosa* and *S. aureus (56, 95, 134)*. For instance, ribosomal proteins associated with metabolic processes were found on the upper layers of the *P. aeruginosa* biofilms (137).

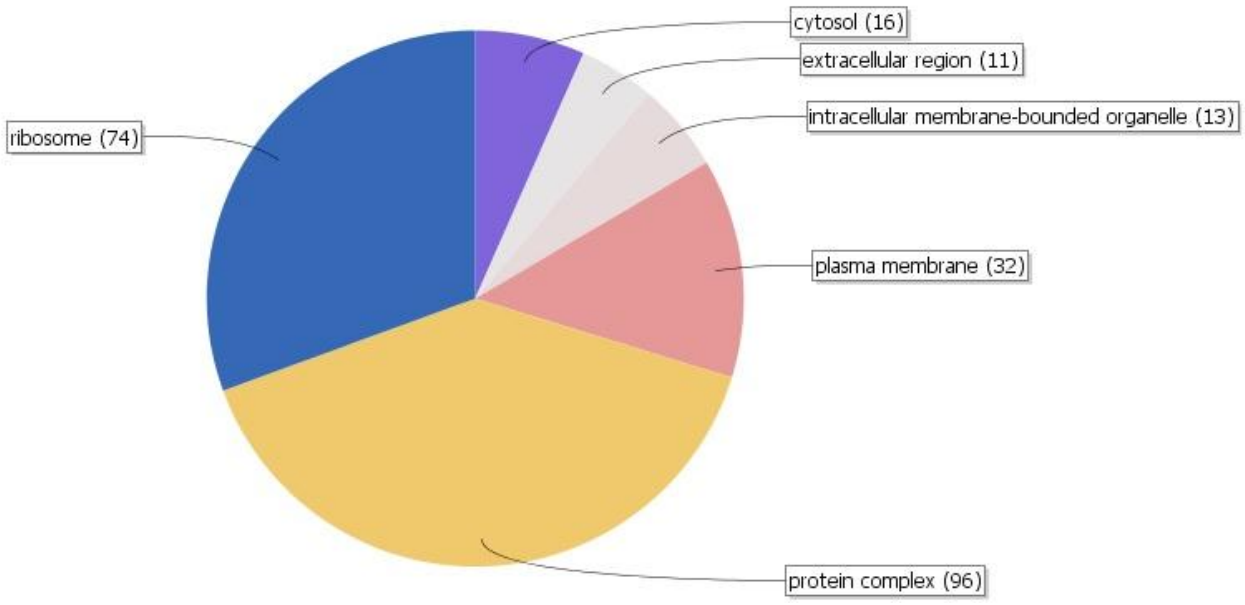

Figure 16- Cellular Component GOs attributed to 10 or more annotated proteins.

The annotated proteins had GOs terms for molecular functions mainly related to binding and enzymatic activity (fig 17). Less prominent functions identified were structural

molecule (of the ribosome) activity and transmembrane transporter activity. Protein binding transcription factor and enzyme regulator activities were also identified but were less common. The highest number of annotated proteins were ion binding proteins, while nucleic acid binding (DNA and rRNA) proteins were less prominent.

As for enzymatic activity GO terms, proteins with oxidoreductase activity were the more common ones followed by transferases, hydrolases, ligases, lyases and isomerases. The annotated hydrolases identified had either ATPase, GTPase, nuclease or carbon-nitrogen (peptide bonds and others) hydrolase activities. On the other hand, the anotated transferases were involved in the transport of acyl, methyl, glycosyl and phosphorous-containing groups (kinases and nucleotidyltransferases).

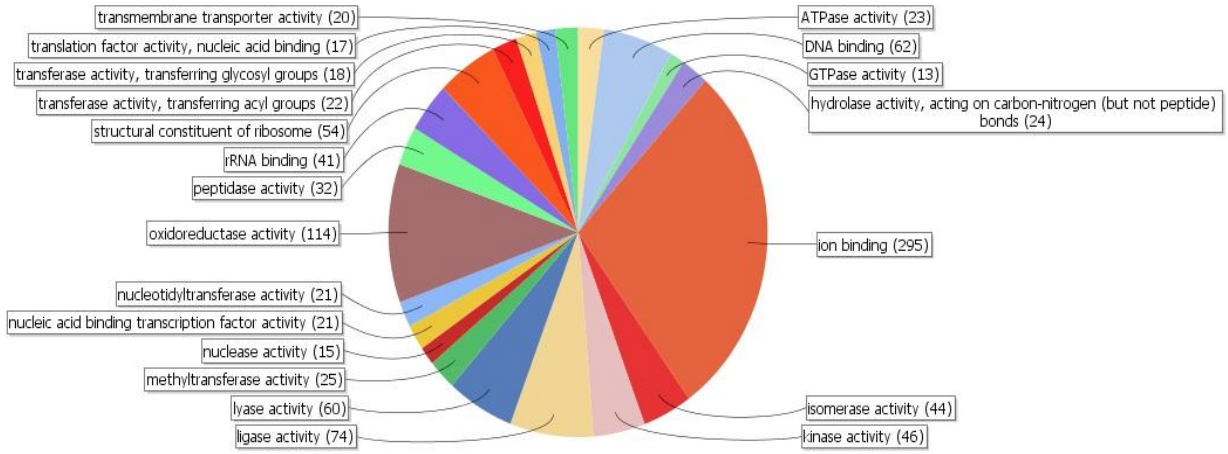

Figure 17- Molecular function GOs attributed to 10 or more annotated proteins.

Most annotated proteins had cell related GOs biological processes such as the cell cycle, cellular division, cell morphogenesis, stress response, cellular component organization and biogenesis (including the cell wall), transmembrane transport, but most notoriously, the cellular metabolic processes, with cellular aminoacid metabolic processes being the most abundant GO (fig 18). Another significant metabolic processes identified were related to protein synthesis, such as ribosome biogenesis and gene translation. Other than this, carbohydrate, lipid and nucleic acid metabolic processes (both DNA and tRNA) also made the threshold.

All of these functions are essential to cell survival and to be expected on a bacterial cell proteome. Although most of these activities are well known intracellular activities, it is not possible to exclude their extracellular presence and their involvement in biofilm

production and maintenance. Indeed, most cellular component GOs are for proteins that are part of protein complexes, but the location of this complexes is not specified. Even if most proteins were of intracellular origin, with the current data it would not be possible to ascertain whether they were normally secreted by biofilm-embedded bacteria or the by-product of cell lysis, either as a regular biofilm function or the result of experimental handling-but that was not the objective of this study.

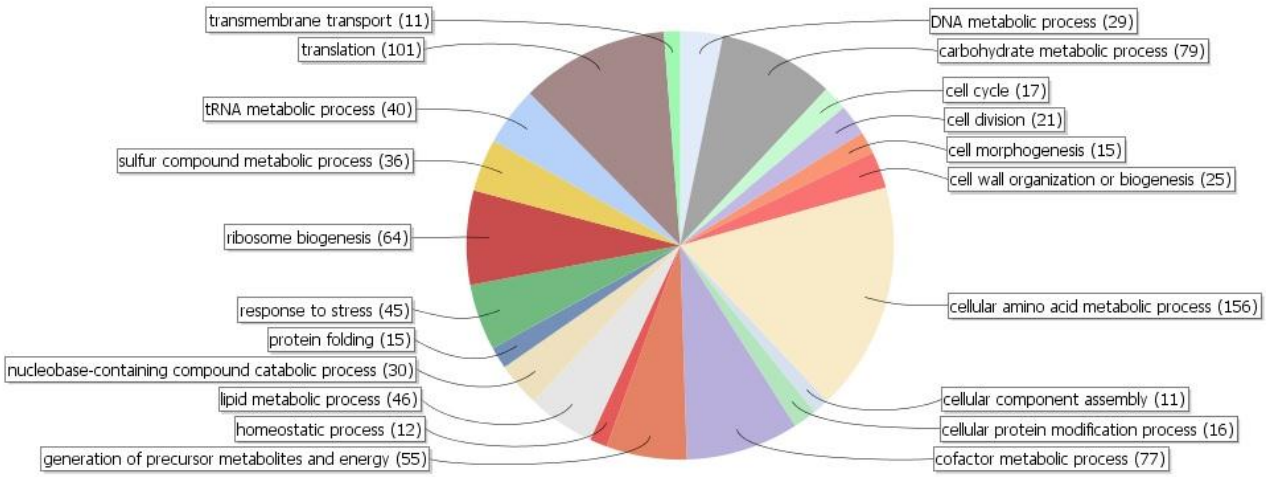

Figure 18- Biological processes GOs attributed to 10 or more annotated proteins.

3.2.2.2 Biofilm-associated proteins: Regulators and Virulence Factors

Planktonic *S. aureus* strains express both *sarA* and *sigB* genes in order to inhibit protease and thermonuclease production that could hinder biofilm production (44-46). Additionally *sigB* stimulates the production of adhesins for the initial covalent attachment of bacteria to a host matrix proteins (48). In the *S. pseudintermedius* 5819/10 biofilm matrix, the presence of the SarA protein was confirmed together with the membrane protein SaeR (fig. 19 and table 6). In *S. aureus*, response regulators SaeR and SaeS act synergistically with SarA to repress extracellular protease production that would otherwise limit accumulation of critical proteins (such as adhesins) that contribute to biofilm formation (138). Sae is required for the induced expression of the important virulence factors IsdA and IsdB in low iron conditions (139). Although these factors were not detected, Clp protease required for *isdB* gene transcription (140) was identified.

Furthermore, SaeR/SaeS activates the expression of exoproteins involved in adhesion and invasion of mammalian host cells, including coagulase, Spa, hemolysins (Hla and Hlb), DNase, and cell wall-associated proteins (Emp, Eap/Map, FnbA) (141-145) as well as the Iron-regulated surface determinant proteins A and B under low iron conditions (139) in order to obtain iron from hemoglobin and other iron-rich host proteins from mammalian host cells (146). Of all of these proteins, only Eap and β-hemolysin (Hlb) were present on the *S. pseudintermedius* 5819/10 biofilm matrix.

SarA and SaeR were not the only DNA-binding proteins responsible for the expression of virulence factors found among the biofilm matrix proteins. The HTH-type transcriptional regulator MgrA, the transcriptional regulatory protein SrrA and the response regulator VraR were also present. In *S. aureus* MgrA, is a repressor of alpha-toxin, coagulase, protease, and protein A as well as an activator of capsular polyssacharide 8 (CP8) and thermonuclease (147). CP8 enhances the antiphagocytic activity of *S. aureus* (148) while thermonuclease is involved in biofilm seeding dispersal (53). The latter was indeed present on the *S. pseudintermedius* 5819/10 biofilm matrix, even with concurrent SarA activity, while the other repressed proteins were not.

SrrA is part of the two-component regulatory system SrrA/SrrB (149), which is involved in the global regulation of staphylococcal virulence factors in response to environmental oxygen levels (59). SrrAB induces *icaADBC* gene transcription and polysaccharide intercellular adhesin expression, protecting *S. aureus* from neutrophil killing under anaerobic growth condition (59). SrrA in itself binds to the Agr, Spa and Toxic shock toxin promoters repressing their transcription under anaerobic conditions but also promotes it under aerobic conditions (149, 150).

VraR, or vancomycin resistance associated regulator is part of the two-component system VraSR, that positively modulates the regulation of cell-wall biosynthesis pathway (in *S. aureus*) (151). Part of the genes regulated by VraSR system are associated with cell-wall biosynthesis, such as PBP2, SgtB and MurZ (152). The sensor kinase VraS has been shown to respond to the damage of cell-wall structure or inhibition of cell-wall biosynthesis by the antimicrobials bacitracin, fosfomycin, teicoplanin, vancomycin and β-lactams, leading to overexpression of VraR and reducing the susceptibility to the said antimicrobials, inhibitors of cell-wall synthesis (151, 152).

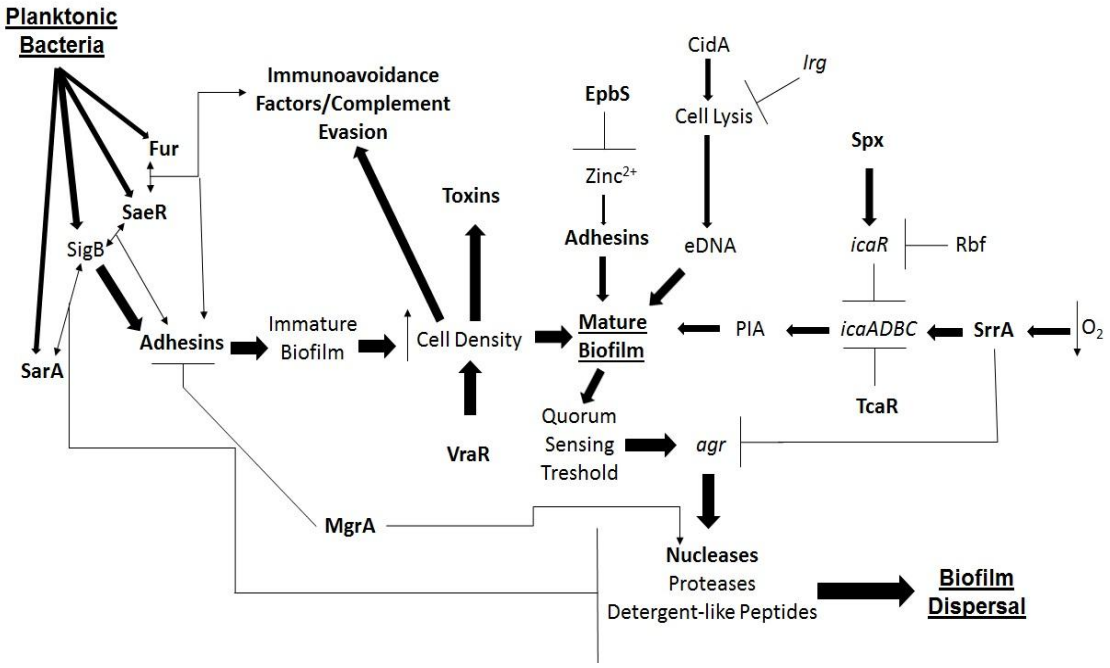

Figure 19- - Proteins involved in the biofilm life cycle present in the S. pseudintermdius 5819/10 biofilm matrix. In bold are proteins that were present in S.pseudintermedius 5819/10 biofilm matrix and in underlined bold are key stages of the biofilm life cycle. Ponty arrows (bar the vertical thin ones close to "O_2" and "cell density" describes the expression of proteins or genes or the ocurrence of an event. T-headed arrows describes the inhibiton of protein or gene expression, the stopaged of cell lysis by Irg gene products as well as zinc-binding by EpbS which prevents SasG-dependent biofilm formation.Two way arrows linked to another describe an action that two proteins execute together; SarA and SaeR also work together to repress extracellular protease production.

RNA polymerase sigma factor SigB was not present in the *S. pseudintermedius* 5819/10 biofilm matrix, nor adherence factors known to be associated with it: clumping factor, fibronectin binding protein A (FnBPA) and coagulase (44, 45). Such proteins bind to human platelets (153) and a serine-rich adhesin for platelets that mediate direct binding to human platelets by *S. aureus* (153) was also present on the *S. pseudintermedius* 5819/10 biofilm matrix. This protein, Serine-rich adhesin for platelets, has been shown to mediate the direct binding of *S. aureus* to human platelets leading to endovascular infection (154).

Eap/Map as well as an autolysin (Atl) were identified. In *S. aureus* MR23 biofilm matrix, Eap/Map was the most abundant protein and was responsible for cell-to-cell aggregation between the bacteria (56). Finding this protein was expected since, both SaeR and a transcriptional regulator from the Fur family were detected. Fur, together with SaeRS are also required for full induction of the oxidative stress response - which is necessary for intracellular survival in neutrophils- and expression of non-covalently bound surface proteins (such as Eap/Map and Emp) in low-iron growth conditions (139).

Regarding the autolysin, the one identified by LC-MS/MS belongs to a different strain of *S. pseudintermedius* but has a 99% similarity to the one from the preliminary assay. In *S. aureus*, this autolysin is a secreted protein required for both FnBP- and PIA-mediated biofilm development on hydrophobic polystyrene and to the attachment to hydrophilic polystyrene (65). Since *S. pseudintermedius* biofilms were grown in polysterene dishes in this experiment, its presence was not unexpected.

Another adhesin associated with biofilm formation found was Elastin binding protein EbpS. It is a weak adhesin though, as inactivation of *ebpS* has a minimal effect on the binding of *S. aureus* to elastin peptides, which in fact is mediated by fibronectin-binding proteins (155). More relevant is its role in the regulation of biofilm formation mechanisms that are zinc concentration dependent (156). In its absence, the addition of $Zinc^{2+}$ ions increases biofilm formation in *S. aureus* (156). Zinc ions are essential for the dimerization of the cell-wall-anchored protein SasG leading to cell-to-cell adhesion (157). It binds to $Zinc^{2+}$ ions, undergoing conformational changes that leads to the formation of aggregates (157). By binding to $Zinc^{2+}$, Ebps regulates the transfer of zinc ions to SasG through competitive binding and thus, also regulates zinc-dependent biofilm formation (156). A homolog of SasG was not found in the *S. pseudintermedius* 5819/10 biofilm matrix though, so there was not enough evidence to support the theory that EpbS regulates Zinc-dependent biofilm formation on this strain.

After adhering to a surface, bacteria start forming EPS either producing polysaccharide intercellular adhesin, teichoic acids, releasing DNA or adhesins. Cell-wall associated adhesins might even work in conjuction with the polyssacarides or DNA, by binding the cells to them such as the autolysin AtlAEfm of *Enterococcus faecium* (158). Adhesins can also bind cells together directly such as the above SasG and Eap. Autolysins also have a role in cell lysis and consequent DNA release (158). In *S. epidermidis*, evidence shows that AtlE mediates cell lysis and thus contributes to biofilm formation (73). However, there was no evidence that the autolysin found on *S. pseudintermedius* 5819/10 biofilm matrix nor its *S. aureus* homolog played a role in eDNA-dependent biofilm formation through cell lysis. The only known protein found to be involved with it, was the thermonuclease mentioned above.

Moreover, despite the presence of SrrA, none of the Ica proteins responsible for PIA synthesis were identified. Indeed, the only proteins associated with PIA synthesis were repressors: the HTH-type transcriptional regulator TcaR and the Regulatory protein Spx that promotes the expression of *icaR* gene and thus the IcaR protein that represses PIA synthesis when binding to the *icaADBC* gene cluster (60). Regarding teichoic acid synthesis, only a glycerol phosphate lipoteichoic acid synthase was identified. This protein synthetizes membrane theicoic acids and thus has a role in bacterial growth and cell division (159) but no evidence supporting a role in biofilm formation was found yet.

Another regulator that is noted for its absence is the accessory gene regulator (*agr*) protein Agr. Agr expression induces the production of nucleases, membrane and cell-wall degrading surfactant-like peptides and proteases that degrade the biofilm matrix and promote seeding dispersion of bacteria (52). Looking at the presented evidence, that was not a surprise. The only protein that could be up-regulated by Agr is thermonuclease. The other proteins identified were associated with biofilm formation and not with bacterial dispersion. SarA, in particular, inhibits the synthesis of said biofilm-associated bacteria dispersion proteins (52).

Finally, as mentioned in section 1.1.3, transient up-regulation of *sarA* and *agr* genes, leads to the production of several immunoavoidance factors and toxins that cause damage to the host organism (52). Though so far associated with those genes, some proteins of this type were indeed found on the *S. pseudintermedius* biofilm matrix. The most infamous protein was the *S.pseudintermedius* exfoliative toxin coded by the *siet* gene as it has been already associated with canine pyoderma (98). Another one found was the synergohymenotropic toxin, which is coded by the luk-I family of genes. Luk-I, which is very similar to Panton–Valentine leucocidin (PVL) from *S. aureus,* shows strong leucotoxicity towards various polymorphonuclear cells.

Although immunoglobulin binding protein A (SpA) was not identified, Immunoglobulin-binding protein Sbi was detected. By binding to IgG-and inactivating it, it allows the bacteria to evade the hosts' immune system (56). There were not tissue damaging proteases apart from Clp in *S. pseudintermedius* biofilm matrix, but a potentially important protease was present: Do-like Serine protease, DegP/HtrA. Its *Streptococcus pyogenes* homolog is involved in the folding and maturation of secreted proteins, as well as in the

degradation of proteins that misfold during secretion (160). Like *S. pseudintermedius*, *Streptococcus pyogenes* is a Gram-positive bacteria and the presence of this protease here, supports the theory that it plays an important role in the biogenesis of secreted proteins in Gram-positive bacteria.

Table 6-Biofilm-associated proteins present on S. pseudintermedius 5819/10 biofilm matrix proteome

Accession Number	Protein Name	Gene Name	Gene Ontology		
			Component	Function	Process
tr|E8SKE3|E8SKE3_STAPH	ATP-dependent Clp protease proteolytic subunit	*clpP*	cytoplasm	hydrolase activity; peptidase activity; serine-type endopeptidase activity; serine-type peptidase activity	proteolysis
tr|F0P7U5|F0P7U5_STAPE	Autolysin	*spsC*	-	amidase activity; hydrolase activity; hydrolase activity, acting on glycosyl bonds; mannosyl-glycoprotein endo-beta-N-acetylglucosaminidase activity; N-acetylmuramoyl-L-alanine amidase activity	metabolic process; peptidoglycan catabolic process
tr|E8SER2|E8SER2_STAPH	Do-like Serine protease, DegP/HtrA	*SPSINT_0718*		catalytic activity; peptidase activity; serine-type endopeptidase activity	proteolysis

tr|E8SHG6|E8SHG6_STAPH	Elastin binding protein EbpS	*SPSINT_11 81*	Cellular component; plasma membrane	-	catabolic process; cell wall organization or biogenesis
tr|F0P3J0|F0P3J0_STAPE	Exfoliative toxin	*siet*	-	peptidase activity	proteolysis
tr|E8SIY6|E8SIY6_STAPH	Extracellular adherence protein of broad specificity Eap/Map	*SPSINT_14 87*	extracellular region	-	pathogenesis
tr|F0P8S4|F0P8S4_STAPE	Glycerol phosphate lipoteichoic acid synthase	*ltaS*	-	catalytic activity; sulfuric ester hydrolase activity; transferase activity	metabolic process
tr|F0P8V5|F0P8V5_STAPE	HTH-type transcriptional regulator MgrA	*mgrA*	intracellular	DNA binding; sequence-specific DNA binding transcription factor activity	regulation of transcription, DNA-templated; transcription, DNA-templated
tr|F0P5W3|F0P5W3_STAPE	HTH-type transcriptional regulator TcaR	*tcaR*	intracellular	sequence-specific DNA binding transcription factor activity	regulation of transcription, DNA-templated
tr|E8SHU1|E8SHU1_STAPH	IgG-binding protein Sbi	*SPSINT_00 32*	-	immunoglobulin binding	pathogenesis
tr|F0P8I4|F0P8I4_STAPE	Regulatory protein Spx	*spxA*	cytoplasm	-	negative regulation of transcription, DNA-templated;regulation of transcription, DNA-templated; transcription, DNA-templated

tr|E8SGR9|E8SGR9_STAPH	Response regulator SaeR	*SPSINT_2275*	-	DNA binding	phosphorelay signal transduction system; regulation of transcription, DNA-templated; transcription, DNA-templated
tr|F0P9I8|F0P9I8_STAPE	Staphylococcal accessory regulator A	*sarA*	Cytoplasm; intracellular	DNA binding; sequence-specific DNA binding transcription factor activity	regulation of transcription, DNA-templated; transcription, DNA-templated
sp|Q5HCP3|SRAP_STAAC	Serine-rich adhesin for platelets	*sraP*	extracellular region; cell wall; membrane	calcium ion binding	pathogenesis
tr|F0P6U1|F0P6U1_STAPE	Synergohymenotropic toxin	*lukF-I*	extracellular region	-	cytolysis in other organism; pathogenesis
tr|F0P6Z1|F0P6Z1_STAPE	Thermonuclease	*nucB*	-	hydrolase activity; hydrolase activity, acting on ester bonds; nucleic acid binding	metabolic process
tr|F0P5I0|F0P5I0_STAPE	Transcriptional regulator, Fur family	*SPSE_1301*	-	DNA binding; sequence-specific DNA binding transcription factor activity	regulation of transcription, DNA-templated
tr|F0P5I7|F0P5I7_STAPE	Transcriptional regulatory protein SrrA	*srrA*	-	DNA binding	phosphorelay signal transduction

					system; regulation of transcription, DNA-templated ; transcription, DNA-templated
tr|F0P648|F0P648_STAPE	Two-component response regulator VraR	*vraR*	-	DNA binding; sequence-specific DNA binding transcription factor activity	phosphorelay signal transduction system; regulation of transcription, DNA-templated; phosphorelay signal transduction system
tr|E8SJ39|E8SJ39_STAPH	β-hemolysin	*SPSINT_0234*	extracellular region	phosphoric diester hydrolase activity	metabolic process

3.2.2.3 Final Remarks

The work developed in this Master Thesis Project showed that the biofilm matrix proteome of a highly virulent *S. pseudintermedius* strain comprised a diverse group of proteins and confirmed the expression of several genome predicted proteins on the biofilm matrix. As in other bacterial BMPs, most of them were related to cellular metabolism. Several were also involved in the metabolism of known components of the cell wall, PIA and teichoic acids such as acetyl-glucosamine, glycerol or phosphate groups. However, it was not possible to establish a clear relation between them and biofilm formation. That was due to the lack of information since the large majority of known *S. pseudintermedius* have been inferred from homology or are just predicted proteins from gene sequences that don't have sufficient information on the database. It would be helpful to perform a detailed function and localization analysis of these proteins in order to better describe the BMP of *S. pseudintermedius* and ascertain the importance of the proteins found on this study.

Proteins known to be involved in biofilm formation consisted mostly of regulator factors of biofilm formation as well as virulence factors of-mainly-bacterial cell adhesion and host colonization. On the other hand, proteins related to PIA-dependent biofilm formation and bacterial seed dispersion were mostly absent. The prevalence of adhesins points to a PIA-independent biofilm where cells are directly or indirectly closely glued together to each other.

In another continuation study, it would be interesting to characterize the composition of all polymers of the biofilm-matrix, particularly regarding its polyssacharides and eDNA composition in order to better understand and evaluate the hypothesis that *S. pseudintermedius* 5819/10 biofilm matrix is mainly constituted by bacteria connected by adhesins and/or eDNA. Additionally, it would be important to find out which virulence factors are more pronounced and their relevance to biofilm formation in order to disrupt them in the future. That way, more information is needed for discovery of anti-biofilm drugs, which is the main goal of the project where this Master Thesis was inserted.

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5.Annexs

5.1 Protein Quantitation Data

Table 7- Calibration Curve Data used for the protein quantitation assay. On the left there's absorbance (Abs) measured for 4 standards and a blank and on the calibration curve parameters follow the y=mX+b equation.

Calibration Curve					
Quantitation Data			Calibration Curve Parameters		
Standards	Mass (µg)	Abs.	m	b	R^2
P1	0	0,886	-0,00738	0,8732	0,994034113
P2	10	0,786			
P3	20	0,721			
P4	30	0,650			
P5	40	0,585			

Table 8- Protein quantitation data for the replicates used in the GeLC-MS/MS analysis. The number in front of the replicate code (ex: PD I2 10 nº) refers to the volume of sample (µL) that was pipetted onto the 96-well plate for measurement.

	Quantitation				
Sample Code	Abs	Mass (µg)	V (µL)	[Protein] (µg/µL)	$[Protein]_{avg}$ (µg/µL)
PD I2 10 5	0,771	13,8	5	2,8	1,9
PD I2 10 10	0,809	8,7	10	0,9	
PD I3 10 5	0,799	10,0	5	2,0	1,8
PD I3 10 10	0,756	15,9	10	1,6	
PD I1 100 5	0,723	20,4	5	4,1	2,8
PD I1 100 10	0,762	15,1	10	1,5	

PD I2 100 5	0,777	13,0	5	2,6	2.1
PD I2 100 10	0,764	14,8	10	1,5	

5.2 LC-MS/MS data from the identified proteins

Table 9-Descritpion of abbreviations, color codes and technical terminology used on table 10.

Color code	
	Directly Identified and annotated proteins
	Blasted and successfully annotated proteins
	Proteins without annotation or identifcation
Abbreviations used (data from ProteinScape 3.1 User Manual and Uniprot Fasta Headers webpage)	
Accession	Database accession name
pI	Isoelectric point of protein
#Alt. Proteins	Number of similar proteins
Scores	Protein score in the format score (M: Mascot score)
#Peptides	Number of peptides identified
SC [%]	Sequence coverage (%)
RMS90 [ppm]	Deviation from predicted mass (root mean square with 90% confidence value)
OS	Scientific name of the organism of the UniProtKB entry
GN	**G**ene **N**ame is the first of the UniprotKB entry
PE	**P**rotein **E**xistence gives a numerical value describing the evidence for the existence of the protein
SV	**S**equence **V**ersion is the version number of the sequence
MW (kDa)	Molecular weight of protein in kilodaltons

Table 10- List of all identified proteins by GeLC-MS/MS.

Accession	Protein	MW [kDa]	pI	#Alt. Proteins	Scores	#Peptides	SC [%]	RMS90 [ppm]
tr|F0P720|F0P720_STAPE	(Dimethylallyl)adenosine tRNA methylthiotransferase MiaB OS=Staphylococcus pseudintermedius (strain ED99) GN=miaB PE=3 SV=1	58,5	5,9	1	77.1 (M:77.1)	2	2,7	4,09
tr|F0P591|F0P591_STAPE	10 kDa chaperonin OS=Staphylococcus pseudintermedius (strain ED99) GN=groS PE=3 SV=1	10,6	5,2	3	344.6 (M:344.6)	5	47,4	4,54
tr|F0P8X4|F0P8X4_STAPE	16s rRNA methyltransferase	22,4	6,9	1	61.7 (M:61.7)	2	16,3	394,47
tr|F0P795|F0P795_STAPE	1-acyl-sn-glycerol-3-phosphate acyltransferase, putative OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1065 PE=4 SV=1	23,2	9,8	2	497.4 (M:497.4)	11	51,7	2,66
tr|F0P7D8|F0P7D8_STAPE	1-deoxy-D-xylulose 5-phosphate reductoisomerase OS=Staphylococcus pseudintermedius (strain ED99) GN=dxr PE=3 SV=1	42,1	5	2	267.4 (M:267.4)	6	17,3	3,27
tr|F0P5F8|F0P5F8_STAPE	1-deoxy-D-xylulose-5-phosphate synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=dxs PE=3 SV=1	68,4	5,8	2	298.0 (M:298.0)	7	12,1	5,46
tr|F0P8L5|F0P8L5_STAPE	1-pyrroline-5-carboxylate dehydrogenase	56,8	5,3	2	224.6 (M:224.6)	5	10,7	402,73
tr|F0P6E0|F0P6E0_STAPE	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=dapD PE=3 SV=1	25,3	4,7	2	115.3 (M:115.3)	2	15,5	272,14
tr|F0P8C3|F0P8C3_STAPE	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase OS=Staphylococcus pseudintermedius (strain ED99) GN=gpmA PE=3 SV=1	26,4	5,6	3	731.0 (M:731.0)	12	63,6	2,47
tr|F0P852|F0P852_STAPE	2,3-bisphosphoglycerate-independent phosphoglycerate mutase OS=Staphylococcus pseudintermedius (strain ED99) GN=gpmI PE=3 SV=1	56,5	4,8	2	817.6 (M:817.6)	15	32,1	3,88
tr|F0P8W4|F0P8W4_STAPE	2-amino-3-ketobutyrate coenzyme A ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=kbl PE=3 SV=1	43,7	5,2	2	891.8 (M:891.8)	18	46,8	120,91
tr|F0P369|F0P369_STAPE	2-dehydropantoate 2-reductase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0212 PE=3 SV=1	34,5	5,3	1	287.6 (M:287.6)	6	23,5	1,83
tr|F0P6C2|F0P6C2_STAPE	2-oxoglutarate dehydrogenase E1 component OS=Staphylococcus pseudintermedius (strain ED99) GN=sucA PE=3 SV=1	104,5	5,2	3	298.4 (M:298.4)	9	12,1	167,93
tr|F0P6C3|F0P6C3_STAPE	2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide succinyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=sucB PE=3 SV=1	46,4	5	3	298.1 (M:298.1)	7	18,8	7

tr|F0P5G5|F0P5G5_STAPE	2-oxoisovalerate dehydrogenase subunit beta OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1284 PE=4 SV=1	35,6	5	1	167.8 (M:167.8)	3	10,7	2,36
tr|F0P6L8|F0P6L8_STAPE	30S ribosomal protein S10 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsJ PE=3 SV=1	11,6	9,7	2	260.3 (M:260.3)	5	33,3	4,64
sp|Q5HDY3|RS11_STAAC	30S ribosomal protein S11 OS=Staphylococcus aureus (strain COL) GN=rpsK PE=3 SV=1	13,9	11,2	2	465.5 (M:465.5)	6	26,4	2,6
tr|F0P4L1|F0P4L1_STAPE	30S ribosomal protein S12 methylthiotransferase	50,8	5,4	2	64.2 (M:64.2)	2	5,1	4,58
tr|F0P8X0|F0P8X0_STAPE	30S ribosomal protein S12 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsL PE=3 SV=1	15,3	11,4	2	152.9 (M:152.9)	4	23,4	6,54
tr|F0P3C0|F0P3C0_STAPE	30S ribosomal protein S13 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsM PE=3 SV=1	13,7	10,3	2	672.9 (M:672.9)	12	59,5	6,24
sp|Q5HDX1|RS14Z_STAAC	30S ribosomal protein S14 type Z OS=Staphylococcus aureus (strain COL) GN=rpsZ PE=3 SV=1	7,3	10,4	2	31.7 (M:31.7)	1	11,5	3,4
sp|Q5HGF8|RS15_STAAC	30S ribosomal protein S15 OS=Staphylococcus aureus (strain COL) GN=rpsO PE=3 SV=1	10,6	10,5	2	50.5 (M:50.5)	2	14,6	5,81
tr|F0P7G8|F0P7G8_STAPE	30S ribosomal protein S16 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsP PE=3 SV=1	10,2	10	2	381.6 (M:381.6)	6	60,4	4,11
tr|F0P6M8|F0P6M8_STAPE	30S ribosomal protein S17 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsQ PE=3 SV=1	10,2	9,8	2	77.8 (M:77.8)	2	20,7	6,78
tr|F0P994|F0P994_STAPE	30S ribosomal protein S18 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsR PE=3 SV=1	9,2	11,2	2	36.1 (M:36.1)	1	10	9,9
tr|F0P6M3|F0P6M3_STAPE	30S ribosomal protein S19 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsS PE=3 SV=1	10,6	10	2	215.9 (M:215.9)	5	35,9	346,75
tr|F0P7E4|F0P7E4_STAPE	30S ribosomal protein S2 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsB PE=3 SV=1	29,9	5,2	2	940.6 (M:940.6)	18	46,8	3,7
tr|F0P6M5|F0P6M5_STAPE	30S ribosomal protein S3 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsC PE=3 SV=1	24,1	9,8	2	590.1 (M:590.1)	11	52,5	2,63
tr|F0P3I3|F0P3I3_STAPE	30S ribosomal protein S4 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsD PE=3 SV=1	22,9	9,9	2	777.2 (M:777.2)	14	52,5	3,72
tr|F0P3B3|F0P3B3_STAPE	30S ribosomal protein S5 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsE PE=3 SV=1	17,7	10,2	2	1033.2 (M:1033.2)	17	69,3	4,72
tr|F0P996|F0P996_STAPE	30S ribosomal protein S6 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsF PE=3 SV=1	11,7	5	2	474.0 (M:474.0)	8	65,3	3,29
tr|F0P8W9|F0P8W9_STAPE	30S ribosomal protein S7 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsG PE=3 SV=1	17,8	10	2	613.9 (M:613.9)	11	50,6	4,76
tr|F0P6N3|F0P6N3_STAPE	30S ribosomal protein S8 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsH PE=3 SV=1	14,8	9,5	2	1102.3 (M:1102.3)	17	67,4	3,63
tr|F0P3C9|F0P3C9_STAPE	30S ribosomal protein S9 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsI PE=3 SV=1	14,9	10,5	2	89.9 (M:89.9)	2	14,4	5,05
tr|F0P916|F0P916_STAPE	33 kDa chaperonin OS=Staphylococcus pseudintermedius (strain ED99) GN=hslO PE=3 SV=1	31,8	4,8	1	230.8 (M:230.8)	6	27,6	2,92
tr|F0P6S1|F0P6S1_STAPE	3'-5' exoribonuclease YhaM OS=Staphylococcus pseudintermedius (strain ED99) GN=cbf1 PE=4 SV=1	35,9	6	2	334.1 (M:334.1)	8	20,1	5,86

tr|F0P680|F0P680_STAPE	3-dehydroquinate synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=aroB PE=3 SV=1	40,6	6,3	2	54.5 (M:54.5)	2	5,6	517,49
tr|F0P7L1|F0P7L1_STAPE	3-demethylubiquinone-9 3-methyltransferase domain protein	15,6	4,7	2	107.1 (M:107.1)	3	19,7	5,02
tr|F0P786|F0P786_STAPE	3-deoxy-7-phosphoheptulonate synthase/chorismate mutase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1056 PE=4 SV=1	40,9	5,8	5	488.1 (M:488.1)	11	23,4	5,37
tr|F0P8D5|F0P8D5_STAPE	3-hexulose-6-phosphate synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=hxlA PE=4 SV=1	22,7	4,6	2	328.0 (M:328.0)	6	34,9	1,63
tr|F0P4D8|F0P4D8_STAPE	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ OS=Staphylococcus pseudintermedius (strain ED99) GN=fabZ PE=3 SV=1	16	5,7	2	208.2 (M:208.2)	5	29,3	4,08
tr|E8SFB9|E8SFB9_STAPH	3-hydroxyacyl-CoA dehydrogenase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2020 PE=4 SV=1	18,8	6,1	2	130.8 (M:130.8)	4	27,2	5,81
tr|E8SI87|E8SI87_STAPH	3-methyl-2-oxobutanoate hydroxymethyltransferase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=panB PE=3 SV=1	29,3	5,1	2	207.0 (M:207.0)	4	14,8	1,06
tr|F0P5Y6|F0P5Y6_STAPE	3-methyladenine dna glycosylase	22,8	9,5	2	73.9 (M:73.9)	2	9,9	927,33
tr|F0P7I6|F0P7I6_STAPE	3-oxoacyl-[acyl-carrier-protein] reductase OS=Staphylococcus pseudintermedius (strain ED99) GN=fabG PE=3 SV=1	26,2	6,1	2	678.4 (M:678.4)	12	55,5	151,5
tr|F0P8J2|F0P8J2_STAPE	3-oxoacyl-[acyl-carrier-protein] synthase 2 OS=Staphylococcus pseudintermedius (strain ED99) GN=fabF PE=3 SV=1	43,5	5	2	640.2 (M:640.2)	10	41,8	255,37
tr|F0P8J3|F0P8J3_STAPE	3-oxoacyl-[acyl-carrier-protein] synthase 3 OS=Staphylococcus pseudintermedius (strain ED99) GN=fabH PE=3 SV=1	34,2	4,8	1	225.9 (M:225.9)	3	13,1	3,07
tr|E8SHF2|E8SHF2_STAPH	3-phosphoshikimate 1-carboxyvinyltransferase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=aroA PE=3 SV=1	46,2	5,4	2	89.1 (M:89.1)	3	6,3	4,21
tr|F0P9J3|F0P9J3_STAPE	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase OS=Staphylococcus pseudintermedius (strain ED99) GN=ispE PE=3 SV=1	30,9	5,2	1	102.6 (M:102.6)	3	13,1	468,52
tr|F0P4N6|F0P4N6_STAPE	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=ispG PE=3 SV=1	39,6	5,6	1	265.3 (M:265.3)	8	26,4	4,61
tr|F0P4N0|F0P4N0_STAPE	4-hydroxy-3-methylbut-2-enyl diphosphate reductase OS=Staphylococcus pseudintermedius (strain ED99) GN=ispH PE=3 SV=1	35,6	5,4	2	113.6 (M:113.6)	3	7,8	5,2
tr|E8SH09|E8SH09_STAPH	4-hydroxy-tetrahydrodipicolinate reductase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=dapB PE=3 SV=1	26,4	5,5	2	122.2 (M:122.2)	3	12,5	5,29
tr|F0P6E2|F0P6E2_STAPE	4-hydroxy-tetrahydrodipicolinate synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=dapA PE=3 SV=1	32,4	4,8	1	167.9 (M:167.9)	3	14,4	1,24
tr|F0P8R7|F0P8R7_STAPE	5 (3)-deoxyribonucleotidase	20,4	4,9	1	180.9 (M:180.9)	3	21,3	1,5
tr|F0P7Y7|F0P7Y7_STAPE	5' nucleotidase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1807 PE=4 SV=1	58,6	5,5	2	61.6 (M:61.6)	2	3	6,41
tr|F0P8X7|F0P8X7_STAPE	50S ribosomal protein L1 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplA PE=3 SV=1	25	9,3	2	1141.0 (M:1141.0)	19	55,8	3,15
tr|F0P8X6|F0P8X6_STAPE	50S ribosomal protein L10 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplJ PE=3 SV=1	17,9	5,2	2	796.8 (M:796.8)	11	60,2	3,69
tr|F0P8X8|F0P8X8_STAPE	50S ribosomal protein L11 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplK PE=3 SV=1	15	9,2	2	440.5 (M:440.5)	8	39,3	3,46

tr|F0P3C8|F0P3C8_STAPE	50S ribosomal protein L13 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplM PE=3 SV=1	16,2	9,3	2	1206.0 (M:1206.0)	19	82,8	4,34
tr|F0P6M9|F0P6M9_STAPE	50S ribosomal protein L14 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplN PE=3 SV=1	13,1	10,2	2	523.7 (M:523.7)	9	50,8	3,96
tr|F0P3B5|F0P3B5_STAPE	50S ribosomal protein L15 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplO PE=3 SV=1	15,6	10,4	2	411.0 (M:411.0)	8	33,6	7,18
tr|F0P6M6|F0P6M6_STAPE	50S ribosomal protein L16 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplP PE=3 SV=1	16,2	10,5	2	292.6 (M:292.6)	5	43,8	5,88
tr|F0P3C3|F0P3C3_STAPE	50S ribosomal protein L17 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplQ PE=3 SV=1	13,7	10	1	269.7 (M:269.7)	4	34,4	2,35
tr|F0P3B2|F0P3B2_STAPE	50S ribosomal protein L18 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplR PE=3 SV=1	13,2	10	1	601.9 (M:601.9)	9	53,8	4,41
tr|F0P6M2|F0P6M2_STAPE	50S ribosomal protein L2 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplB PE=3 SV=1	30,3	10,7	1	46.9 (M:46.9)	2	7,2	4,31
tr|F0P3M6|F0P3M6_STAPE	50S ribosomal protein L20 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplT PE=3 SV=1	13,6	11,2	2	252.7 (M:252.7)	6	41,5	3,83
tr|F0P3P6|F0P3P6_STAPE	50S ribosomal protein L21 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplU PE=3 SV=1	11,4	9,8	2	647.9 (M:647.9)	10	76,5	1,74
tr|F0P6M4|F0P6M4_STAPE	50S ribosomal protein L22 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplV PE=3 SV=1	12,9	10,1	2	458.1 (M:458.1)	8	70,1	5,15
tr|F0P6M1|F0P6M1_STAPE	50S ribosomal protein L23 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplW PE=3 SV=1	10,6	9,8	2	431.6 (M:431.6)	8	61,5	4,33
tr|F0P6N0|F0P6N0_STAPE	50S ribosomal protein L24 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplX PE=3 SV=1	11,4	9,9	1	246.7 (M:246.7)	4	23,1	5,5
tr|F0P927|F0P927_STAPE	50S ribosomal protein L25 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplY PE=3 SV=1	23,6	4,3	2	479.3 (M:479.3)	7	28,7	1,33
tr|F0P3P8|F0P3P8_STAPE	50S ribosomal protein L27 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpmA PE=3 SV=1	10,3	10,2	2	219.0 (M:219.0)	4	39,4	3,43
tr|F0P7J5|F0P7J5_STAPE	50S ribosomal protein L28 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpmB PE=3 SV=1	7	12	2	64.7 (M:64.7)	2	27,4	9,16
tr|F0P6M7|F0P6M7_STAPE	50S ribosomal protein L29 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpmC PE=3 SV=1	8	9,5	2	275.9 (M:275.9)	4	55,1	1,33
tr|F0P6L9|F0P6L9_STAPE	50S ribosomal protein L3 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplC PE=3 SV=1	23,7	9,7	2	682.3 (M:682.3)	14	45,7	112,52
tr|F0P3B4|F0P3B4_STAPE	50S ribosomal protein L30 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpmD PE=3 SV=1	6,4	10,1	2	329.9 (M:329.9)	6	74,6	5,11
tr|F0P4B6|F0P4B6_STAPE	50S ribosomal protein L31 type B OS=Staphylococcus pseudintermedius (strain ED99) GN=rpmE PE=3 SV=1	9,8	6,7	2	835.8 (M:835.8)	13	86,9	5,71
tr|F0P3B9|F0P3B9_STAPE	50S ribosomal protein L36 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpmJ PE=3 SV=1	4,3	11,4	2	60.7 (M:60.7)	2	54,1	636,92
tr|F0P6M0|F0P6M0_STAPE	50S ribosomal protein L4 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplD PE=3 SV=1	22,7	9,9	2	440.3 (M:440.3)	8	27,1	2,69
tr|F0P6N1|F0P6N1_STAPE	50S ribosomal protein L5 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplE PE=3 SV=1	20,2	9,5	2	1611.8 (M:1611.8)	24	83,8	2,86

tr|F0P6N4|F0P6N4_STAPE	50S ribosomal protein L6 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplF PE=3 SV=1	19,6	9,1	1	985.1 (M:985.1)	15	51,1	3,34
tr|F0P8X5|F0P8X5_STAPE	50S ribosomal protein L7/L12 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplL PE=3 SV=1	12,5	4,5	2	638.3 (M:638.3)	12	92,6	4,74
tr|F0P4P7|F0P4P7_STAPE	50S ribosomal protein L9 OS=Staphylococcus pseudintermedius (strain ED99) GN=rplI PE=3 SV=1	16,4	9,4	2	388.4 (M:388.4)	7	42,6	4,25
tr|E8SJB3|E8SJB3_STAPH	6,7-dimethyl-8-ribityllumazine synthase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=ribH PE=3 SV=1	16,4	6,1	2	410.3 (M:410.3)	7	44,5	2,38
tr|F0P592|F0P592_STAPE	60 kDa chaperonin OS=Staphylococcus pseudintermedius (strain ED99) GN=groL PE=3 SV=1	57,6	4,5	24	2265.3 (M:2265.3)	37	65,1	5,4
tr|E8SIY2|E8SIY2_STAPH	6'-aminoglycoside N-acetyltransferase (AAC(6')) / 2''-aminoglycoside phosphotransferase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1483 PE=4 SV=1	56,8	4,6	1	179.9 (M:179.9)	6	9,2	6,2
tr|F0P8D4|F0P8D4_STAPE	6-phospho 3-hexuloisomerase OS=Staphylococcus pseudintermedius (strain ED99) GN=hxlB PE=4 SV=1	19,5	4,8	2	117.3 (M:117.3)	2	12,1	7,27
tr|F0P3K7|F0P3K7_STAPE	6-phosphofructokinase OS=Staphylococcus pseudintermedius (strain ED99) GN=pfkA PE=3 SV=1	34,9	5,4	2	736.3 (M:736.3)	13	34,8	3,24
tr|F0P5H0|F0P5H0_STAPE	6-phosphogluconate dehydrogenase, decarboxylating OS=Staphylococcus pseudintermedius (strain ED99) GN=gnd PE=3 SV=1	51,7	4,9	2	1372.4 (M:1372.4)	26	57,8	76,68
tr|E8SK67|E8SK67_STAPH	6-phosphogluconolactonase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1674 PE=4 SV=1	39	5	3	479.1 (M:479.1)	11	31,8	163,38
tr|F0P9D0|F0P9D0_STAPE	ABC transporter ATP-binding protein VraD OS=Staphylococcus pseudintermedius (strain ED99) GN=vraD PE=4 SV=1	28,1	8,9	1	90.2 (M:90.2)	3	18,6	269,29
tr|F0P5V9|F0P5V9_STAPE	ABC transporter, permease protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0448 PE=4 SV=1	39	5,8	1	72.6 (M:72.6)	3	9,3	3,43
tr|F0P418|F0P418_STAPE	ABC transporter, substrate-binding protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0275 PE=4 SV=1	55,5	5,1	2	169.0 (M:169.0)	4	10,6	312,69
tr|E8SIW7|E8SIW7_STAPH	Acetate kinase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=ackA PE=3 SV=1	44,1	5,3	4	1375.4 (M:1375.4)	22	59	5,3
tr|F0P4I5|F0P4I5_STAPE	acetyl- biotin carboxyl carrier protein	16,8	4,6	1	114.6 (M:114.6)	2	20,3	2,72
tr|F0P5F2|F0P5F2_STAPE	Acetyl-CoA carboxylase, biotin carboxylase subunit OS=Staphylococcus pseudintermedius (strain ED99) GN=accC PE=4 SV=1	50,4	5,1	2	168.8 (M:168.8)	5	10,6	435,57
tr|E8SIV5|E8SIV5_STAPH	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=accA PE=3 SV=1	35,2	5,3	3	181.8 (M:181.8)	4	14,6	4,78
tr|F0P3K5|F0P3K5_STAPE	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta OS=Staphylococcus pseudintermedius (strain ED99) GN=accD PE=3 SV=1	31,9	6	2	388.6 (M:388.6)	10	31,8	4,69
tr|F0P9S4|F0P9S4_STAPE	acetyltransferase	9,9	6	2	97.2 (M:97.2)	2	18,7	5,25
tr|F0P520|F0P520_STAPE	Acetyltransferase, GNAT family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0392 PE=4 SV=1	21,1	4,7	1	125.9 (M:125.9)	4	22,7	5,05
tr|F0P8J5|F0P8J5_STAPE	Acid phosphatase 5'-nucleotidase, lipoprotein e(P4) family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1859 PE=4 SV=1	34,1	8,9	1	161.6 (M:161.6)	3	10	2,96
tr|F0P6W1|F0P6W1_STAPE	Aconitate hydratase 1 OS=Staphylococcus pseudintermedius (strain ED99) GN=acnA PE=4 SV=1	98,4	4,7	3	1201.0 (M:1201.0)	27	31,5	5,54

tr|F0P7I5|F0P7I5_STAPE	Acyl carrier protein OS=Staphylococcus pseudintermedius (strain ED99) GN=acpP PE=3 SV=1	8,5	3,8	2	69.3 (M:69.3)	2	31,2	4,46
tr|B1GVE3|B1GVE3_9STAP	acyl dehydratase	16,1	5,4	1	67.4 (M:67.4)	2	19,7	1,67
tr|F0P6D2|F0P6D2_STAPE	Acylphosphatase OS=Staphylococcus pseudintermedius (strain ED99) GN=acyP PE=3 SV=1	10,3	5,6	2	130.1 (M:130.1)	3	36,7	6,89
tr|F0P3Q8|F0P3Q8_STAPE	Adenine phosphoribosyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=apt PE=3 SV=1	19	4,8	1	80.1 (M:80.1)	2	12,8	1,21
tr|F0P3B7|F0P3B7_STAPE	Adenylate kinase OS=Staphylococcus pseudintermedius (strain ED99) GN=adk PE=3 SV=1	24,2	4,9	2	784.4 (M:784.4)	15	54,4	3,82
tr|F0P616|F0P616_STAPE	Adenylosuccinate lyase OS=Staphylococcus pseudintermedius (strain ED99) GN=purB PE=4 SV=1	49,6	5,8	1	153.8 (M:153.8)	4	8,8	466,55
tr|E8SHP3|E8SHP3_STAPH	Adenylosuccinate synthetase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=purA PE=3 SV=1	47,4	5,2	4	468.8 (M:468.8)	9	20,6	4,53
tr|F0P5H9|F0P5H9_STAPE	ADP-ribose pyrophosphatase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1300 PE=3 SV=1	20,5	5,4	1	378.2 (M:378.2)	7	37,8	384,96
tr|E8SGK6|E8SGK6_STAPH	Aerobic glycerol-3-phosphate dehydrogenase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1034 PE=3 SV=1	62	7,7	3	1009.9 (M:1009.9)	18	33,5	4,82
tr|T2I4H1|T2I4H1_9STAP	Alanine dehydrogenase OS=Staphylococcus pseudintermedius GN=ald1 PE=4 SV=1	39,8	5,4	2	654.4 (M:654.4)	11	28,6	4,24
tr|F0P549|F0P549_STAPE	Alanine racemase OS=Staphylococcus pseudintermedius (strain ED99) GN=alrA PE=3 SV=1	42,7	5,2	2	170.8 (M:170.8)	4	13,1	259,15
tr|E8SIG8|E8SIG8_STAPH	Alanine--tRNA ligase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=alaS PE=3 SV=1	98,4	5,1	3	818.5 (M:818.5)	19	21,9	232,16
tr|E8SFE2|E8SFE2_STAPH	Alcohol dehydrogenase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2043 PE=4 SV=1	36	6,3	2	411.4 (M:411.4)	7	27,2	3,78
tr|F0P4B3|F0P4B3_STAPE	aldehyde dehydrogenase	52	5	2	1650.6 (M:1650.6)	25	69,2	63,36
tr|F0P5Z1|F0P5Z1_STAPE	aldose 1-epimerase	38,5	4,9	1	166.6 (M:166.6)	4	9,7	7,03
tr|F0P3L2|F0P3L2_STAPE	Alkaline phosphatase synthesis transcriptional regulatory protein OS=Staphylococcus pseudintermedius (strain ED99) GN=phoP PE=4 SV=1	27,2	5,1	2	147.0 (M:147.0)	3	17,9	490,69
tr|F0P3F3|F0P3F3_STAPE	Alkaline shock protein 23 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0630 PE=4 SV=1	18,3	5,2	2	927.4 (M:927.4)	15	81,6	5,3
tr|F0P984|F0P984_STAPE	Alkyl hydroperoxide reductase subunit C OS=Staphylococcus pseudintermedius (strain ED99) GN=ahpC PE=4 SV=1	21	4,6	2	774.9 (M:774.9)	11	66,1	4,35
tr|F0P985|F0P985_STAPE	alkyl hydroperoxide reductase subunit f	54,5	4,7	2	304.0 (M:304.0)	7	23,7	140,73
tr|E8SGB7|E8SGB7_STAPH	Alkylphosphonate utilization operon protein PhnA OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2205 PE=4 SV=1	12,7	4,8	2	134.3 (M:134.3)	3	34,5	321,22
tr|F0P4I4|F0P4I4_STAPE	Allophanate hydrolase subunit 2 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1185 PE=4 SV=1	38	9	1	53.5 (M:53.5)	2	5,9	8,19
tr|F0P703|F0P703_STAPE	Aluminum resistance protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1465 PE=4 SV=1	45,8	5,1	2	96.2 (M:96.2)	2	4,6	4,45

tr|F0P601|F0P601_STAPE	Amidohydrolase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0490 PE=4 SV=1	42,4	5	1	117.2 (M:117.2)	3	6,6	4,24
tr|F0P3Y0|F0P3Y0_STAPE	Amidophosphoribosyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=purF PE=3 SV=1	52,2	5,8	1	47.0 (M:47.0)	2	5,5	4,68
tr|F0P6F0|F0P6F0_STAPE	Aminoacyltransferase FemA OS=Staphylococcus pseudintermedius (strain ED99) GN=femA PE=4 SV=1	49,5	6,8	2	165.6 (M:165.6)	5	11,6	393,95
tr|F0P6E9|F0P6E9_STAPE	Aminoacyltransferase FemB OS=Staphylococcus pseudintermedius (strain ED99) GN=femB PE=4 SV=1	49,3	5,9	2	281.1 (M:281.1)	7	16,9	2,49
tr|G4XUK6|G4XUK6_9STAP	Aminoglycoside 3-phosphotransferase OS=Staphylococcus pseudintermedius GN=aphA-3 PE=4 SV=1	30,6	4,5	2	332.1 (M:332.1)	8	26,1	4,95
tr|F0P654|F0P654_STAPE	Aminopeptidase Peps OS=Staphylococcus pseudintermedius (strain ED99) GN=pepS PE=4 SV=1	45,9	4,7	2	320.4 (M:320.4)	7	21,2	3,71
tr|F0P357|F0P357_STAPE	Anaerobic ribonucleoside-triphosphate reductase OS=Staphylococcus pseudintermedius (strain ED99) GN=nrdD PE=4 SV=1	70,4	5,5	1	90.5 (M:90.5)	2	3,2	4,63
tr|F0P554|F0P554_STAPE	Anti-sigma B factor OS=Staphylococcus pseudintermedius (strain ED99) GN=rsbW PE=4 SV=1	18,2	4,8	2	265.1 (M:265.1)	4	30,6	2,36
tr|F0P553|F0P553_STAPE	Anti-sigma factor antagonist OS=Staphylococcus pseudintermedius (strain ED99) GN=rsbV PE=3 SV=1	12,2	4,8	1	128.0 (M:128.0)	3	21,3	5
tr|E8SE31|E8SE31_STAPH	Arginase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1826 PE=3 SV=1	33	4,9	2	152.0 (M:152.0)	4	14	6,13
tr|F0P5F9|F0P5F9_STAPE	Arginine repressor OS=Staphylococcus pseudintermedius (strain ED99) GN=argR PE=3 SV=1	17,2	5,2	2	387.4 (M:387.4)	6	48	2,73
tr|F0P879|F0P879_STAPE	Arginine--tRNA ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=argS PE=3 SV=1	62,6	5,3	3	563.2 (M:563.2)	15	31	215,12
tr|F0P829|F0P829_STAPE	arsenate reductase	13,7	6,1	1	441.7 (M:441.7)	10	62,2	5,69
tr|F0P691|F0P691_STAPE	Asparagine--tRNA ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=asnS PE=3 SV=1	49,3	5,2	2	634.0 (M:634.0)	14	32,1	4,53
tr|E8SK80|E8SK80_STAPH	Aspartate aminotransferase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1687 PE=4 SV=1	48,1	4,9	2	102.1 (M:102.1)	2	4,7	7,45
tr|F0P7L9|F0P7L9_STAPE	Aspartate carbamoyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=pyrB PE=3 SV=1	33,1	5,2	1	103.1 (M:103.1)	2	7,2	5,03
tr|F0P6E3|F0P6E3_STAPE	Aspartate-semialdehyde dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=asd PE=3 SV=1	35,8	5	2	100.0 (M:100.0)	2	6,4	4,09
tr|F0P4G2|F0P4G2_STAPE	Aspartate--tRNA ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=aspS PE=3 SV=1	66,3	4,9	2	1110.4 (M:1110.4)	21	33,8	4,79
tr|F0P6Y3|F0P6Y3_STAPE	Aspartokinase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1444 PE=3 SV=1	50,5	6,1	2	151.7 (M:151.7)	5	11,1	283,96
tr|F0P626|F0P626_STAPE	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B OS=Staphylococcus pseudintermedius (strain ED99) GN=gatB PE=3 SV=1	53,3	5	2	837.6 (M:837.6)	19	40,4	5
tr|F0P4D4|F0P4D4_STAPE	ATP synthase epsilon chain OS=Staphylococcus pseudintermedius (strain ED99) GN=atpC PE=3 SV=1	15	5,6	1	274.7 (M:274.7)	4	35,8	1,56
tr|F0P4D2|F0P4D2_STAPE	ATP synthase gamma chain OS=Staphylococcus pseudintermedius (strain ED99) GN=atpG PE=3 SV=1	32,3	8,5	2	710.1 (M:710.1)	13	50,5	3,01

tr|F0P4D1|F0P4D1_STAPE	ATP synthase subunit alpha OS=Staphylococcus pseudintermedius (strain ED99) GN=atpA PE=3 SV=1	54,7	4,9	2	1249.7 (M:1249.7)	21	43,7	3,1
tr|F0P4C9|F0P4C9_STAPE	ATP synthase subunit b OS=Staphylococcus pseudintermedius (strain ED99) GN=atpF PE=3 SV=1	19,4	5,4	2	262.0 (M:262.0)	5	25,6	5,51
tr|F0P4D3|F0P4D3_STAPE	ATP synthase subunit beta OS=Staphylococcus pseudintermedius (strain ED99) GN=atpD PE=3 SV=1	51,2	4,7	2	2044.8 (M:2044.8)	34	79,4	99,84
tr|E8SKP1|E8SKP1_STAPH	ATP synthase subunit delta OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=atpH PE=3 SV=1	20,2	5,4	2	151.7 (M:151.7)	3	17,9	704,77
tr|F0P900|F0P900_STAPE	atp:guanido phosphotransferase	38,1	5,1	2	428.2 (M:428.2)	7	21,6	4,22
tr|F0P3H1|F0P3H1_STAPE	ATP-binding protein, Mrp/Nbp35 family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0648 PE=4 SV=1	38,5	5,3	3	127.1 (M:127.1)	3	9	322,51
tr|F0P8J6|F0P8J6_STAPE	ATP-dependent chaperone protein ClpB OS=Staphylococcus pseudintermedius (strain ED99) GN=clpB PE=3 SV=1	98,7	5,2	2	395.2 (M:395.2)	10	12,3	5,62
tr|F0P8Z9|F0P8Z9_STAPE	ATP-dependent Clp protease ATP-binding subunit ClpC OS=Staphylococcus pseudintermedius (strain ED99) GN=clpC PE=3 SV=1	91,2	5,4	2	630.8 (M:630.8)	13	19,5	4,92
tr|F0P3N0|F0P3N0_STAPE	ATP-dependent Clp protease ATP-binding subunit ClpX OS=Staphylococcus pseudintermedius (strain ED99) GN=clpX PE=3 SV=1	46,2	4,5	2	477.0 (M:477.0)	8	28,6	265,02
tr|E8SKE3|E8SKE3_STAPH	ATP-dependent Clp protease proteolytic subunit OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=clpP PE=3 SV=1	21,4	5	2	492.7 (M:492.7)	9	26,2	1,74
tr|E8SK52|E8SK52_STAPH	ATP-dependent DNA helicase UvrD/PcrA OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1659 PE=4 SV=1	83,9	5,5	3	134.7 (M:134.7)	3	5,2	4,19
tr|F0P7E6|F0P7E6_STAPE	ATP-dependent protease ATPase subunit HslU OS=Staphylococcus pseudintermedius (strain ED99) GN=hslU PE=3 SV=1	53	5,3	2	689.5 (M:689.5)	15	35,2	148,51
tr|E8SIP7|E8SIP7_STAPH	ATP-dependent zinc metalloprotease FtsH OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=ftsH PE=3 SV=1	78	5,2	3	509.6 (M:509.6)	11	15,5	5,37
tr|F0P7U5|F0P7U5_STAPE	Autolysin OS=Staphylococcus pseudintermedius (strain ED99) GN=spsC PE=4 SV=1	150,5	9,3	2	1953.5 (M:1953.5)	42	33,4	5,2
tr|F0P8E1|F0P8E1_STAPE	bacillithiol biosynthesis deacetylase 2	25,3	4,7	2	236.0 (M:236.0)	5	20,3	3,54
tr|F0P4G8|F0P4G8_STAPE	Bacterial luciferase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1169 PE=4 SV=1	38	5,8	2	163.3 (M:163.3)	3	9,5	1,18
tr|F0P396|F0P396_STAPE	Betaine aldehyde dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=betB PE=3 SV=1	54,8	5	3	814.3 (M:814.3)	18	50,1	91,18
tr|F0P9Q8|F0P9Q8_STAPE	Beta-lactamase OS=Staphylococcus pseudintermedius (strain ED99) GN=blaZ-2 PE=4 SV=1	31,3	9,6	2	172.1 (M:172.1)	4	19,2	2,58
tr|F0P3Y7|F0P3Y7_STAPE	Bifunctional protein FolD OS=Staphylococcus pseudintermedius (strain ED99) GN=folD PE=3 SV=1	30,9	5,1	2	489.2 (M:489.2)	8	29,8	5,64
tr|F0P929|F0P929_STAPE	Bifunctional protein GlmU OS=Staphylococcus pseudintermedius (strain ED99) GN=glmU PE=3 SV=1	48,5	5,8	3	443.7 (M:443.7)	8	30,4	257,44
tr|F0P7M1|F0P7M1_STAPE	Bifunctional protein PyrR OS=Staphylococcus pseudintermedius (strain ED99) GN=pyR PE=3 SV=1	19,6	5,2	2	134.0 (M:134.0)	3	16,7	5,12
tr|F0P3X7|F0P3X7_STAPE	Bifunctional purine biosynthesis protein PurH OS=Staphylococcus pseudintermedius (strain ED99) GN=purH PE=3 SV=1	53,8	5,3	2	880.0 (M:880.0)	14	38	166,46

tr|F0P8F1|F0P8F1_STAPE	Branched-chain-amino-acid aminotransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=ilvE PE=3 SV=1	40,2	4,9	3	223.1 (M:223.1)	5	15,8	5,81
tr|E8SEX0|E8SEX0_STAPH	Butyryl-CoA dehydrogenase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1953 PE=4 SV=1	43	5,5	2	211.8 (M:211.8)	5	15,3	370,73
tr|F0P727|F0P727_STAPE	Cadmium efflux system accessory protein OS=Staphylococcus pseudintermedius (strain ED99) GN=cadC PE=4 SV=1	14,4	6,3	1	73.4 (M:73.4)	2	11,1	4,78
tr|F0P7L6|F0P7L6_STAPE	Carbamoyl-phosphate synthase large chain OS=Staphylococcus pseudintermedius (strain ED99) GN=carB PE=3 SV=1	116,6	5	2	1156.5 (M:1156.5)	25	20,9	6,33
tr|F0P7L7|F0P7L7_STAPE	Carbamoyl-phosphate synthase small chain OS=Staphylococcus pseudintermedius (strain ED99) GN=carA PE=3 SV=1	40,1	5,6	1	449.7 (M:449.7)	9	29,5	3,62
tr|F0P649|F0P649_STAPE	carbonic anhydrase	21,5	5	2	95.5 (M:95.5)	3	17,6	372,36
tr|F0P848|F0P848_STAPE	Carboxylesterase OS=Staphylococcus pseudintermedius (strain ED99) GN=est PE=4 SV=1	28,1	4,7	1	70.6 (M:70.6)	2	8,9	598,53
tr|F0P787|F0P787_STAPE	Catabolite control protein A OS=Staphylococcus pseudintermedius (strain ED99) GN=ccpA PE=4 SV=1	36,5	5,7	2	637.5 (M:637.5)	14	38	3,75
tr|F0P5Q2|F0P5Q2_STAPE	Catalase OS=Staphylococcus pseudintermedius (strain ED99) GN=katA PE=3 SV=1	57	5,6	2	1390.1 (M:1390.1)	24	58,3	99,03
tr|E8SIB7|E8SIB7_STAPH	CBS domain protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1332 PE=4 SV=1	18,4	8,5	3	72.5 (M:72.5)	2	11,7	3,58
tr|F0P7M5|F0P7M5_STAPE	cell division protein DivIVA	23,1	4,9	2	458.4 (M:458.4)	9	39,7	2,94
tr|F0P7P0|F0P7P0_STAPE	Cell division protein ftsA OS=Staphylococcus pseudintermedius (strain ED99) GN=ftsA PE=3 SV=1	52,1	4,5	1	688.3 (M:688.3)	14	33,3	166,05
tr|E8SJ87|E8SJ87_STAPH	Cell division protein FtsK OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1508 PE=4 SV=1	122,6	5,2	3	93.5 (M:93.5)	2	2,7	7,39
tr|F0P7P6|F0P7P6_STAPE	Cell division protein FtsL OS=Staphylococcus pseudintermedius (strain ED99) GN=ftsL PE=3 SV=1	14,9	9,6	1	65.4 (M:65.4)	1	10,2	4,15
tr|F0P7N9|F0P7N9_STAPE	Cell division protein FtsZ OS=Staphylococcus pseudintermedius (strain ED99) GN=ftsZ PE=3 SV=1	40,9	4,9	3	1503.7 (M:1503.7)	18	50	4,48
sp|Q5HGP2|SEPF_STAAC	Cell division protein SepF OS=Staphylococcus aureus (strain COL) GN=sepF PE=3 SV=1	21	4,9	2	124.5 (M:124.5)	3	15	2,7
tr|E8SIR2|E8SIR2_STAPH	Cell shape-determining protein MreC OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1413 PE=3 SV=1	31	7,2	1	142.7 (M:142.7)	4	20,5	319,32
tr|F0P4Y8|F0P4Y8_STAPE	Cell wall biosynthesis protein ScdA OS=Staphylococcus pseudintermedius (strain ED99) GN=scdA PE=3 SV=1	25,3	5,3	1	285.7 (M:285.7)	6	32,1	2,41
tr|E8SK62|E8SK62_STAPH	Cell wall surface anchor family protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1669 PE=4 SV=1	40,9	6,5	2	115.3 (M:115.3)	3	6,8	5,69
tr|E8SID3|E8SID3_STAPH	Chaperone protein DnaJ OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=dnaJ PE=3 SV=1	41,6	6,4	3	218.9 (M:218.9)	6	21,5	4,25
tr|F0P4K7|F0P4K7_STAPE	Chaperone protein DnaK OS=Staphylococcus pseudintermedius (strain ED99) GN=dnaK PE=3 SV=1	66	4,6	3	2327.1 (M:2327.1)	38	63,8	75,52
tr|F0P3Y9|F0P3Y9_STAPE	Chitinase-related protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1733 PE=4 SV=1	11,8	4,4	1	108.3 (M:108.3)	3	28,8	2

tr|F0P679|F0P679_STAPE	Chorismate synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=aroC PE=3 SV=1	42,8	5,9	2	290.5 (M:290.5)	6	17,2	3,31
tr|E8SHQ9|E8SHQ9_STAPH	Chromosomal replication initiator protein DnaA OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=dnaA PE=3 SV=1	51,1	5,3	3	224.5 (M:224.5)	6	10,5	6,33
tr|F0P9T3|F0P9T3_STAPE	Chromosome partioning protein, ParB family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2499 PE=4 SV=1	32,6	5,4	1	210.8 (M:210.8)	5	18,4	273,74
tr|F0P3K9|F0P3K9_STAPE	Citrate synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=gltA PE=3 SV=1	42	5,3	2	229.7 (M:229.7)	6	16,4	5,56
tr|F0P8K1|F0P8K1_STAPE	Coenzyme A disulfide reductase OS=Staphylococcus pseudintermedius (strain ED99) GN=cdr PE=4 SV=1	49,6	5,9	2	361.7 (M:361.7)	8	17	4,35
tr|F0P8E5|F0P8E5_STAPE	Cof-like hydrolase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2223 PE=4 SV=1	31,7	5	2	364.3 (M:364.3)	6	28,8	4,28
tr|F0P8L8|F0P8L8_STAPE	Cold shock protein B OS=Staphylococcus pseudintermedius (strain ED99) GN=cspB PE=3 SV=1	7,3	4,5	2	251.4 (M:251.4)	4	60,6	3,81
tr|F0P6D4|F0P6D4_STAPE	Cold shock protein OS=Staphylococcus pseudintermedius (strain ED99) GN=cspA PE=3 SV=1	7,3	4,5	3	257.0 (M:257.0)	5	50	237,65
tr|F0P6E5|F0P6E5_STAPE	Conserved virulence factor B OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1398 PE=4 SV=1	34,9	5,1	1	124.0 (M:124.0)	3	10,6	2,88
tr|F0P6A7|F0P6A7_STAPE	Conserved virulence factor C OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1357 PE=4 SV=1	42,6	5,1	1	121.1 (M:121.1)	3	9,7	3,59
tr|F0P4A6|F0P4A6_STAPE	CTP synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=pyrG PE=3 SV=1	60,2	5	2	533.1 (M:533.1)	11	27,2	4,84
tr|F0P9M7|F0P9M7_STAPE	Cystathionine gamma-synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=metB2 PE=3 SV=1	41,6	4,9	2	327.3 (M:327.3)	6	15	4,26
tr|F0P817|F0P817_STAPE	Cysteine desulfurase OS=Staphylococcus pseudintermedius (strain ED99) GN=sufS PE=3 SV=1	45,9	5,1	2	370.8 (M:370.8)	7	22,3	3,23
tr|F0P915|F0P915_STAPE	Cysteine synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=cysK PE=4 SV=1	33	5,5	2	1855.8 (M:1855.8)	25	77	2,16
tr|F0P9M8|F0P9M8_STAPE	Cysteine synthase/cystathionine beta-synthase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2356 PE=4 SV=1	32,8	4,9	2	306.2 (M:306.2)	7	26,9	266,52
tr|F0P8Y6|F0P8Y6_STAPE	Cysteine--tRNA ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=cysS PE=3 SV=1	53,7	5	2	296.9 (M:296.9)	6	13,7	3,44
tr|F0P8U3|F0P8U3_STAPE	Cys-tRNA(Pro)/Cys-tRNA(Cys) deacylase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2046 PE=3 SV=1	17,6	7,2	1	132.3 (M:132.3)	3	10,1	8,14
tr|F0P4M0|F0P4M0_STAPE	Cytidine deaminase OS=Staphylococcus pseudintermedius (strain ED99) GN=cdd PE=4 SV=1	14,8	4,9	1	390.3 (M:390.3)	6	49,6	4,27
tr|F0P5J6|F0P5J6_STAPE	Cytidylate kinase OS=Staphylococcus pseudintermedius (strain ED99) GN=cmk PE=3 SV=1	24,6	4,8	2	319.8 (M:319.8)	6	32	2,08
tr|F0P8N3|F0P8N3_STAPE	cytosol aminopeptidase	54,4	5,8	1	89.6 (M:89.6)	3	6,1	348,23
tr|F0P797|F0P797_STAPE	D-3-phosphoglycerate dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=serA PE=4 SV=1	57,7	5,3	1	215.7 (M:215.7)	4	12,1	231,37
tr|F0P7J3|F0P7J3_STAPE	dak2 domain fusion protein	60,7	4,6	3	699.1 (M:699.1)	13	26,2	3,97

tr|F0P773|F0P773_STAPE	D-alanine aminotransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=dat PE=3 SV=1	31,6	5,1	1	1022.1 (M:1022.1)	16	72	2,77
tr|F0P4F5|F0P4F5_STAPE	D-alanine--D-alanine ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=ddl PE=3 SV=1	39,9	5	2	404.7 (M:404.7)	10	31,5	5,13
tr|E8SER8|E8SER8_STAPH	D-alanine--poly(Phosphoribitol) ligase subunit 1 OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0724 PE=3 SV=1	54,4	5,5	2	286.8 (M:286.8)	6	12,6	5,1
tr|F0P7Z1|F0P7Z1_STAPE	D-alanine--poly(phosphoribitol) ligase subunit 2 OS=Staphylococcus pseudintermedius (strain ED99) GN=dltC PE=3 SV=1	9	3,9	2	39.7 (M:39.7)	1	19,2	4,63
tr|E8SIE2|E8SIE2_STAPH	dCMP deaminase Late competence protein ComEB OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1357 PE=3 SV=1	17,2	6,5	3	165.8 (M:165.8)	3	18,3	5,29
tr|F0P4F7|F0P4F7_STAPE	dead deah box helicase	56,1	9,5	1	45.5 (M:45.5)	2	5,1	314,5
tr|E8SGZ9|E8SGZ9_STAPH	Deblocking aminopeptidase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1096 PE=4 SV=1	38,1	5,4	2	95.5 (M:95.5)	3	7,3	5,95
tr|F0P8W1|F0P8W1_STAPE	Decarboxylase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2064 PE=4 SV=1	20,9	5,2	3	123.2 (M:123.2)	3	20,1	6,3
tr|F0P6B1|F0P6B1_STAPE	DegV family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1361 PE=4 SV=1	31,2	6,2	2	525.6 (M:525.6)	9	32	3,62
tr|F0P3N6|F0P3N6_STAPE	Delta-aminolevulinic acid dehydratase OS=Staphylococcus pseudintermedius (strain ED99) GN=hemB PE=3 SV=1	36,5	5,1	2	210.2 (M:210.2)	6	22,5	1,43
tr|F0P5K2|F0P5K2_STAPE	Demethylmenaquinone methyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=ubiE PE=3 SV=1	26,9	8,6	2	215.5 (M:215.5)	6	18,1	1,89
tr|F0P8E8|F0P8E8_STAPE	DeoxyadenosIne kinase OS=Staphylococcus pseudintermedius (strain ED99) GN=dak PE=4 SV=1	26,3	5,5	3	89.4 (M:89.4)	2	12,2	369
tr|F0P447|F0P447_STAPE	Deoxycytidine triphosphate deaminase OS=Staphylococcus pseudintermedius (strain ED99) GN=dcd PE=3 SV=1	20,2	4,8	1	131.8 (M:131.8)	3	23,2	1,66
tr|F0P3A8|F0P3A8_STAPE	Deoxyribose-phosphate aldolase OS=Staphylococcus pseudintermedius (strain ED99) GN=deoC PE=3 SV=1	23,7	4,9	2	625.2 (M:625.2)	11	61,4	128,27
tr|F0P3L6|F0P3L6_STAPE	Dephospho-CoA kinase OS=Staphylococcus pseudintermedius (strain ED99) GN=coaE PE=3 SV=1	23,1	4,9	1	461.8 (M:461.8)	9	46,9	2,36
tr|F0P3K2|F0P3K2_STAPE	DHH subfamily phosphodiesterase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1098 PE=4 SV=1	34,8	4,6	2	48.8 (M:48.8)	2	6,5	5
tr|E8SH13|E8SH13_STAPH	Diaminopimelate decarboxylase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=lysA PE=3 SV=1	46,7	5,3	2	150.7 (M:150.7)	4	8,6	370,56
tr|E8SH41|E8SH41_STAPH	Dihydrofolate reductase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1138 PE=3 SV=1	18,3	5	2	142.4 (M:142.4)	3	23,3	5,73
tr|E8SHJ4|E8SHJ4_STAPH	Dihydrolipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase complex OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1209 PE=3 SV=1	46,9	5,2	2	108.1 (M:108.1)	3	5,2	4,15
tr|F0P3V2|F0P3V2_STAPE	Dihydrolipoyl dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=pdhD PE=3 SV=1	49,5	4,8	1	1969.1 (M:1969.1)	29	62	4,33
tr|F0P7L8|F0P7L8_STAPE	Dihydroorotase OS=Staphylococcus pseudintermedius (strain ED99) GN=pyrC PE=3 SV=1	46,1	5,1	3	460.5 (M:460.5)	11	36,3	4,63
tr|F0P7L5|F0P7L5_STAPE	Dihydroorotate dehydrogenase B (NAD(+)), electron transfer subunit OS=Staphylococcus pseudintermedius (strain ED99) GN=pyrK PE=3 SV=1	27,6	5,2	1	269.0 (M:269.0)	6	24	2,05

tr|F0P7L4|F0P7L4_STAPE	Dihydroorotate dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=pyrD PE=3 SV=1	32,7	5,6	1	105.5 (M:105.5)	2	9,8	4,36
tr|F0P914|F0P914_STAPE	Dihydropteroate synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=folP PE=4 SV=1	29,4	4,9	1	120.8 (M:120.8)	2	6,7	2,24
tr|F0P402|F0P402_STAPE	Dihydroxyacetone kinase, DhaK subunit OS=Staphylococcus pseudintermedius (strain ED99) GN=dhaK PE=4 SV=1	34,9	4,7	3	414.5 (M:414.5)	8	24,1	5,36
tr|F0P403|F0P403_STAPE	Dihydroxyacetone kinase, DhaL subunit OS=Staphylococcus pseudintermedius (strain ED99) GN=dhaL PE=4 SV=1	21,1	4,5	1	659.2 (M:659.2)	9	52,8	5,28
tr|E8SI70|E8SI70_STAPH	Dipeptide-binding ABC transporter, periplasmic substrate-binding component OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0079 PE=4 SV=1	59,9	5,9	2	118.8 (M:118.8)	3	6,9	5,37
tr|E8SDR2|E8SDR2_STAPH	D-lactate dehydrogenase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0522 PE=4 SV=1	37	4,7	2	95.6 (M:95.6)	3	6,3	5,09
tr|B0B0K7|B0B0K7_9STAP	DNA gyrase subunit B OS=Staphylococcus pseudintermedius GN=gyrB PE=3 SV=1	68,9	5,7	5	339.0 (M:339.0)	10	13,9	5,45
tr|F0P621|F0P621_STAPE	DNA ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=ligA PE=3 SV=1	75	5,2	3	168.5 (M:168.5)	4	7,8	5,27
tr|E8SGK1|E8SGK1_STAPH	DNA mismatch repair protein MutL OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=mutL PE=3 SV=1	73,3	5,2	2	118.2 (M:118.2)	4	5,7	667,87
tr|E8SGK0|E8SGK0_STAPH	DNA mismatch repair protein MutS OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=mutS PE=3 SV=1	98,3	5	2	131.1 (M:131.1)	4	5,2	334,4
tr|E8SHQ8|E8SHQ8_STAPH	DNA polymerase III subunit beta OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2450 PE=3 SV=1	41,8	4,7	3	352.0 (M:352.0)	6	16	4,67
tr|E8SIU8|E8SIU8_STAPH	DNA polymerase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1449 PE=3 SV=1	99,2	5	3	681.6 (M:681.6)	15	18,4	4,04
tr|F0P7D1|F0P7D1_STAPE	DNA-binding protein	11	9,5	1	26.9 (M:26.9)	1	11,6	1,85
tr|F0P977|F0P977_STAPE	dna-binding protein	23,6	5,2	2	502.2 (M:502.2)	7	44,7	5,69
tr|F0P5K0|F0P5K0_STAPE	DNA-binding protein HU OS=Staphylococcus pseudintermedius (strain ED99) GN=hup PE=3 SV=1	9,6	9,5	2	989.5 (M:989.5)	11	73,3	150,12
tr|F0P9C0|F0P9C0_STAPE	dna-binding regulatory family	26,3	4,6	2	119.8 (M:119.8)	3	11,3	0,69
tr|F0P3C2|F0P3C2_STAPE	DNA-directed RNA polymerase subunit alpha OS=Staphylococcus pseudintermedius (strain ED99) GN=rpoA PE=3 SV=1	35	4,7	2	566.2 (M:566.2)	11	32,2	4,35
tr|F0P8X3|F0P8X3_STAPE	DNA-directed RNA polymerase subunit beta OS=Staphylococcus pseudintermedius (strain ED99) GN=rpoB PE=3 SV=1	133,5	5	10	1356.7 (M:1356.7)	28	24,4	5,32
tr|E8SDZ9|E8SDZ9_STAPH	dna-directed rna polymerase subunit delta	21,2	3,6	3	209.3 (M:209.3)	4	24,6	2,54
tr|F0P7K8|F0P7K8_STAPE	DNA-directed RNA polymerase subunit omega OS=Staphylococcus pseudintermedius (strain ED99) GN=rpoZ PE=3 SV=1	7,9	7	1	65.5 (M:65.5)	1	13	5,41
tr|E8SJ75|E8SJ75_STAPH	Do-like Serine protease, DegP/HtrA OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1496 PE=4 SV=1	45,1	5,8	2	754.7 (M:754.7)	11	30,5	4,23
tr|F0P7N4|F0P7N4_STAPE	D-ribose pyranase OS=Staphylococcus pseudintermedius (strain ED99) GN=rbsD PE=3 SV=1	15	5,7	3	85.3 (M:85.3)	2	12,7	4,98

tr|E8SHG6|E8SHG6_STAPH	Elastin binding protein EbpS OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1181 PE=4 SV=1	49	5,5	2	386.9 (M:386.9)	7	19,1	5,26
tr|F0P4K3|F0P4K3_STAPE	Elongation factor 4 OS=Staphylococcus pseudintermedius (strain ED99) GN=lepA PE=3 SV=1	68,1	5	2	49.5 (M:49.5)	2	3,1	2,14
tr|F0P8W8|F0P8W8_STAPE	Elongation factor G OS=Staphylococcus pseudintermedius (strain ED99) GN=fusA PE=3 SV=1	76,7	4,8	2	1882.5 (M:1882.5)	36	52,7	4,21
tr|F0P5F0|F0P5F0_STAPE	Elongation factor P OS=Staphylococcus pseudintermedius (strain ED99) GN=efp PE=3 SV=1	20,6	4,9	2	361.5 (M:361.5)	7	30,3	3,63
tr|F0P7E3|F0P7E3_STAPE	Elongation factor Ts OS=Staphylococcus pseudintermedius (strain ED99) GN=tsf PE=3 SV=1	32,4	5,2	2	1804.0 (M:1804.0)	26	74	2,41
tr|F0P8W7|F0P8W7_STAPE	Elongation factor Tu OS=Staphylococcus pseudintermedius (strain ED99) GN=tuf PE=3 SV=1	43,2	4,7	17	2612.3 (M:2612.3)	34	78,5	3,41
tr|E8SIA9|E8SIA9_STAPH	endonuclease iv	33,2	5,7	3	507.7 (M:507.7)	9	31,6	3,81
tr|E8SEL4|E8SEL4_STAPH	Endonuclease/Exonuclease/phosphatase family protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1927 PE=4 SV=1	69,4	8,5	2	436.7 (M:436.7)	11	19,6	5,18
tr|F0P931|F0P931_STAPE	endoribonuclease L-PSP	14	5	1	40.3 (M:40.3)	1	11,9	1,38
tr|F0P851|F0P851_STAPE	Enolase OS=Staphylococcus pseudintermedius (strain ED99) GN=eno PE=3 SV=1	47	4,5	2	2072.1 (M:2072.1)	28	75,6	82,35
tr|F0P8G8|F0P8G8_STAPE	Enoyl-[acyl-carrier-protein] reductase [NADPH] OS=Staphylococcus pseudintermedius (strain ED99) GN=fabI PE=3 SV=1	27,8	5,9	2	610.8 (M:610.8)	12	40	151,32
tr|E8SIK3|E8SIK3_STAPH	Excinuclease ABC OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0130 PE=4 SV=1	83,8	6	2	156.0 (M:156.0)	4	5,7	388,7
tr|F0P3J0|F0P3J0_STAPE	Exfoliative toxin OS=Staphylococcus pseudintermedius (strain ED99) GN=siet PE=4 SV=1	35,8	5,3	2	477.6 (M:477.6)	7	31,2	173,54
tr|E8SIY6|E8SIY6_STAPH	Extracellular adherence protein of broad specificity Eap/Map OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1487 PE=4 SV=1	27,4	9,8	2	1587.8 (M:1587.8)	23	64	3,34
tr|E8SHG9|E8SHG9_STAPH	Ferredoxin OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1184 PE=4 SV=1	8,9	3,5	2	93.1 (M:93.1)	2	17,1	529,77
tr|F0P722|F0P722_STAPE	Ferredoxin oxidoreductase subunit beta OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1484 PE=4 SV=1	31,1	5	2	468.0 (M:468.0)	8	37,8	3,52
tr|E8SEW8|E8SEW8_STAPH	Ferrichrome-binding periplasmic protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1951 PE=4 SV=1	34,5	5,5	2	163.4 (M:163.4)	4	20,5	368,45
tr|F0P640|F0P640_STAPE	Ferritin OS=Staphylococcus pseudintermedius (strain ED99) GN=ftnA PE=4 SV=1	19,9	4,6	2	223.3 (M:223.3)	3	20,4	543,46
tr|F0P6T3|F0P6T3_STAPE	Ferrochelatase OS=Staphylococcus pseudintermedius (strain ED99) GN=hemH PE=3 SV=1	35,4	4,9	1	376.2 (M:376.2)	7	27,7	1,4
tr|Q5HHH2|Q5HHH2_STAAC	FeS assembly ATPase SufC OS=Staphylococcus aureus (strain COL) GN=SACOL0914 PE=3 SV=1	28,3	4,9	2	75.1 (M:75.1)	2	13,4	277,55
tr|F0P815|F0P815_STAPE	FeS assembly protein SufB OS=Staphylococcus pseudintermedius (strain ED99) GN=sufB PE=4 SV=1	52,5	5,1	2	420.2 (M:420.2)	11	23,2	5,72
tr|F0P9S7|F0P9S7_STAPE	flavin reductase	23	4,8	1	114.2 (M:114.2)	2	10,2	1,52

tr|F0P3Y8|F0P3Y8_STAPE	Flavohemoprotein OS=Staphylococcus pseudintermedius (strain ED99) GN=hmp PE=3 SV=1	42,3	5,4	1	472.5 (M:472.5)	10	24,1	2,97
tr|F0P5P5|F0P5P5_STAPE	FMN-dependent NADH-azoreductase OS=Staphylococcus pseudintermedius (strain ED99) GN=azoR PE=3 SV=1	23,2	5,1	1	746.5 (M:746.5)	12	55,8	4,88
tr|F0P6S2|F0P6S2_STAPE	Foldase protein PrsA OS=Staphylococcus pseudintermedius (strain ED99) GN=prsA PE=4 SV=1	36,3	6,3	2	1053.3 (M:1053.3)	21	59,1	3,66
tr|F0P4Z1|F0P4Z1_STAPE	Formate acetyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=pflB PE=3 SV=1	84,7	5,4	2	2233.2 (M:2233.2)	38	39	4,53
tr|F8TLV0|F8TLV0_9STAP	Formate dehydrogenase OS=Staphylococcus pseudintermedius PE=3 SV=1	110,2	5,2	3	217.2 (M:217.2)	7	7,6	373,93
tr|F0P791|F0P791_STAPE	Formate--tetrahydrofolate ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=fhs PE=3 SV=1	60,2	5,9	4	385.9 (M:385.9)	8	13,3	5,09
tr|F0P432|F0P432_STAPE	Fructosamine kinase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0289 PE=4 SV=1	32,4	4,4	2	224.5 (M:224.5)	3	13,9	4,91
tr|F0P4B0|F0P4B0_STAPE	Fructose-1,6-bisphosphate aldolase, class II OS=Staphylococcus pseudintermedius (strain ED99) GN=fba PE=4 SV=1	31,2	5,1	3	1064.7 (M:1064.7)	20	50,3	3,91
tr|E8SGQ2|E8SGQ2_STAPH	Fructose-bisphosphate aldolase class I OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2258 PE=4 SV=1	33	4,9	2	1963.0 (M:1963.0)	33	92,6	125,44
tr|E8SJR0|E8SJR0_STAPH	Fumarate hydratase class II OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=fumC PE=3 SV=1	50,8	5,4	3	297.9 (M:297.9)	8	20,8	5,29
tr|F0P8K4|F0P8K4_STAPE	Fumarylacetoacetate hydrolase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1868 PE=4 SV=1	33,1	4,9	2	921.6 (M:921.6)	18	68,1	95,61
tr|F0P3I4|F0P3I4_STAPE	GAF domain protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1080 PE=4 SV=1	16,2	4,6	2	253.7 (M:253.7)	4	31,8	2,19
tr|E8SEV7|E8SEV7_STAPH	Galactose-1-phosphate uridylyltransferase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=galT PE=3 SV=1	57,1	5,1	2	57.9 (M:57.9)	2	3,8	3,83
tr|F0P5D2|F0P5D2_STAPE	Glucokinase OS=Staphylococcus pseudintermedius (strain ED99) GN=glk PE=4 SV=1	35,3	5,3	1	238.0 (M:238.0)	4	15,2	2,74
tr|F0P8D6|F0P8D6_STAPE	Glucosamine-6-phosphate deaminase OS=Staphylococcus pseudintermedius (strain ED99) GN=nagB PE=3 SV=1	27,3	4,8	2	72.2 (M:72.2)	2	8,2	1,96
tr|F0P6Q5|F0P6Q5_STAPE	Glucosamine-6-phosphate isomerase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0945 PE=4 SV=1	22,2	5,9	1	649.9 (M:649.9)	10	41,7	164,33
tr|F0P5H4|F0P5H4_STAPE	Glucose-6-phosphate 1-dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=zwf PE=3 SV=1	57,1	5,4	3	787.8 (M:787.8)	17	40,7	3,1
tr|F0P8K9|F0P8K9_STAPE	Glucose-6-phosphate isomerase OS=Staphylococcus pseudintermedius (strain ED99) GN=pgi PE=3 SV=1	49,2	4,9	2	1008.9 (M:1008.9)	18	62,5	4,05
tr|F0P8L3|F0P8L3_STAPE	Glutamate dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=gudB PE=3 SV=1	45,5	5,1	2	399.8 (M:399.8)	10	25,1	3,69
tr|F0P3N7|F0P3N7_STAPE	Glutamate-1-semialdehyde 2,1-aminomutase OS=Staphylococcus pseudintermedius (strain ED99) GN=hemL PE=3 SV=1	46,7	4,8	2	630.8 (M:630.8)	11	35,3	2,07
tr|E8SJ07|E8SJ07_STAPH	Glutamate--tRNA ligase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=gltX PE=3 SV=1	56,2	5	2	337.8 (M:337.8)	7	15,7	276,86
tr|F0P642|F0P642_STAPE	glutamine amidotransferase	27	5,6	1	62.0 (M:62.0)	2	7,5	976,53

tr|F0P906|F0P906_STAPE	Glutamine amidotransferase subunit PdxT OS=Staphylococcus pseudintermedius (strain ED99) GN=pdxT PE=3 SV=1	20,6	5,2	2	244.0 (M:244.0)	6	44,9	197,45
tr|F0P701|F0P701_STAPE	Glutamine synthetase OS=Staphylococcus pseudintermedius (strain ED99) GN=glnA PE=3 SV=1	51	5,1	2	965.0 (M:965.0)	18	51,1	4,02
tr|F0P3I0|F0P3I0_STAPE	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] OS=Staphylococcus pseudintermedius (strain ED99) GN=glmS PE=3 SV=1	66,1	5	3	1358.8 (M:1358.8)	25	37	5,57
tr|F0P778|F0P778_STAPE	Glutamyl aminopeptidase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1048 PE=4 SV=1	39,3	5,3	2	116.7 (M:116.7)	2	6,5	2,88
tr|F0P625|F0P625_STAPE	Glutamyl-tRNA(Gln) amidotransferase subunit A OS=Staphylococcus pseudintermedius (strain ED99) GN=gatA PE=3 SV=1	53	5	2	633.4 (M:633.4)	11	25,7	3,1
tr|F0P705|F0P705_STAPE	Glutathione peroxidase OS=Staphylococcus pseudintermedius (strain ED99) GN=bsaA PE=3 SV=1	17,8	6,5	3	493.0 (M:493.0)	13	71,3	4,4
tr|F0P855|F0P855_STAPE	Glyceraldehyde-3-phosphate dehydrogenase 1 OS=Staphylococcus pseudintermedius (strain ED99) GN=gapA PE=3 SV=1	36,4	4,8	24	1495.1 (M:1495.1)	22	54,9	5,3
tr|F0P3L7|F0P3L7_STAPE	Glyceraldehyde-3-phosphate dehydrogenase 2 OS=Staphylococcus pseudintermedius (strain ED99) GN=gapB PE=3 SV=1	36,8	5,4	1	64.1 (M:64.1)	2	7,1	3,08
tr|F0P712|F0P712_STAPE	Glycerol kinase OS=Staphylococcus pseudintermedius (strain ED99) GN=glpK PE=3 SV=1	55,8	4,9	2	692.0 (M:692.0)	12	24,8	4,45
tr|F0P8S4|F0P8S4_STAPE	Glycerol phosphate lipoteichoic acid synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=ltaS PE=4 SV=1	74,3	6,3	3	366.5 (M:366.5)	9	17,4	3,15
tr|F0P715|F0P715_STAPE	Glycerol uptake operon antiterminator regulatory protein OS=Staphylococcus pseudintermedius (strain ED99) GN=glpP PE=4 SV=1	19,9	7,1	2	158.2 (M:158.2)	3	24,3	3,2
tr|F0P9F3|F0P9F3_STAPE	Glycerol-3-phosphate cytidylyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=tagD PE=4 SV=1	15,8	5,4	2	416.5 (M:416.5)	10	47,7	4,1
tr|F0P5J9|F0P5J9_STAPE	Glycerol-3-phosphate dehydrogenase [NAD(P)+] OS=Staphylococcus pseudintermedius (strain ED99) GN=gpsA PE=3 SV=1	35,9	5,7	1	152.8 (M:152.8)	4	13,9	280,43
tr|F0P3S6|F0P3S6_STAPE	glycerophosphoryl diester phosphodiesterase	34,9	8	2	161.0 (M:161.0)	4	14,5	3,02
tr|B1GVE4|B1GVE4_9STAP	Glycerophosphoryl diester phosphodiesterase OS=Staphylococcus pseudintermedius GN=ugpQ PE=4 SV=1	27,9	4,9	1	232.3 (M:232.3)	6	28,3	3,89
tr|F0P828|F0P828_STAPE	Glycine cleavage system H protein OS=Staphylococcus pseudintermedius (strain ED99) GN=gcvH PE=3 SV=1	13,8	4	2	254.9 (M:254.9)	4	50	282,08
tr|F0P5E4|F0P5E4_STAPE	glycine dehydrogenase subunit 2	54,9	5,4	2	47.9 (M:47.9)	2	4,3	488,6
tr|E8SIB8|E8SIB8_STAPH	Glycine--tRNA ligase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=glyQS PE=3 SV=1	53,5	5	3	1155.7 (M:1155.7)	20	54,1	4,83
tr|F0P856|F0P856_STAPE	Glycolytic operon regulator OS=Staphylococcus pseudintermedius (strain ED99) GN=gapR PE=4 SV=1	38,7	8,4	1	180.5 (M:180.5)	5	19,1	490,04
tr|F0P687|F0P687_STAPE	Glycosyl transferase, group 1 family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1335 PE=4 SV=1	42,2	5,4	3	102.4 (M:102.4)	3	8	6,92
tr|F0P9S6|F0P9S6_STAPE	Glyoxylase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2492 PE=4 SV=1	36,5	4,8	2	604.1 (M:604.1)	10	37,2	4,02
tr|F0P802|F0P802_STAPE	Glyoxylate reductase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1904 PE=4 SV=1	36,1	5,3	1	356.8 (M:356.8)	7	22,1	5,07

tr|F0P6X4|F0P6X4_STAPE	GMP reductase OS=Staphylococcus pseudintermedius (strain ED99) GN=guaC PE=3 SV=1	35,9	6,5	4	398.4 (M:398.4)	10	31,4	3,9
tr|F0P971|F0P971_STAPE	GMP synthase [glutamine-hydrolyzing] OS=Staphylococcus pseudintermedius (strain ED99) GN=guaA PE=3 SV=1	58,4	4,9	2	526.4 (M:526.4)	13	24,8	136,41
tr|E8SJ47|E8SJ47_STAPH	GTP cyclohydrolase FolE2 OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=folE2 PE=3 SV=1	33,4	5,3	3	191.7 (M:191.7)	4	13,7	4,22
tr|F0P5J8|F0P5J8_STAPE	GTPase Der OS=Staphylococcus pseudintermedius (strain ED99) GN=engA PE=3 SV=1	49	5,7	2	527.7 (M:527.7)	10	24,8	4,94
tr|F0P4M1|F0P4M1_STAPE	GTPase Era OS=Staphylococcus pseudintermedius (strain ED99) GN=era PE=3 SV=1	34,1	6,4	1	61.2 (M:61.2)	2	8,7	2,29
tr|F0P3P9|F0P3P9_STAPE	GTPase obg OS=Staphylococcus pseudintermedius (strain ED99) GN=obgE PE=3 SV=1	47,4	5,2	1	214.5 (M:214.5)	5	14,2	4,1
tr|F0P9A5|F0P9A5_STAPE	GTP-binding protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2477 PE=4 SV=1	40,5	4,7	2	447.1 (M:447.1)	9	32,1	132,82
tr|F0P7E5|F0P7E5_STAPE	GTP-sensing transcriptional pleiotropic repressor CodY OS=Staphylococcus pseudintermedius (strain ED99) GN=codY PE=3 SV=1	28,6	5,6	1	840.7 (M:840.7)	15	48,6	2,91
tr|F0P7K9|F0P7K9_STAPE	Guanylate kinase OS=Staphylococcus pseudintermedius (strain ED99) GN=gmk PE=3 SV=1	24	5,4	1	107.5 (M:107.5)	2	10,6	1,53
tr|F0P4J5|F0P4J5_STAPE	HAD family hydrolase	22,4	5,7	1	94.7 (M:94.7)	2	8,8	6,62
tr|F0P803|F0P803_STAPE	HAD-superfamily subfamily IIA hydrolase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1905 PE=4 SV=1	28,3	4,4	2	56.0 (M:56.0)	2	7,7	508,43
tr|F0P608|F0P608_STAPE	haloacid dehalogenase-like family hydrolase	23,8	5,1	2	86.1 (M:86.1)	2	12,1	367,17
tr|F0P4K5|F0P4K5_STAPE	Heat-inducible transcription repressor hrcA OS=Staphylococcus pseudintermedius (strain ED99) GN=hrcA PE=3 SV=1	37,3	6,1	2	322.7 (M:322.7)	7	26,1	3,87
tr|E8SGW7|E8SGW7_STAPH	heat-shock protein	16,8	5,3	2	377.0 (M:377.0)	7	62,5	154,08
tr|F0P4E9|F0P4E9_STAPE	heavy metal-associated domain protein	8,1	4,6	1	24.2 (M:24.2)	1	21,9	8,46
tr|E8SGB4|E8SGB4_STAPH	Heme-degrading cytoplasmic oxygenase IsdI OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2202 PE=4 SV=1	12,8	8	2	21.7 (M:21.7)	1	11	2,78
tr|F0P6S7|F0P6S7_STAPE	Histidine triad (HIT) protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0967 PE=4 SV=1	15,9	5	3	399.8 (M:399.8)	8	51,8	2,68
tr|F0P4G1|F0P4G1_STAPE	Histidine--tRNA ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=hisS PE=3 SV=1	48,4	5,4	3	321.2 (M:321.2)	8	20,1	3,48
tr|F0P8R8|F0P8R8_STAPE	Histidinol-phosphate aminotransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=hisC PE=3 SV=1	39	5,1	2	237.6 (M:237.6)	6	20,3	4,88
tr|F0P3Q2|F0P3Q2_STAPE	Holliday junction ATP-dependent DNA helicase RuvB OS=Staphylococcus pseudintermedius (strain ED99) GN=ruvB PE=3 SV=1	37,7	5,1	2	50.0 (M:50.0)	2	4,5	5,74
tr|F0P4H5|F0P4H5_STAPE	Holliday junction resolvase	15,7	5,1	2	89.9 (M:89.9)	2	12,7	3,28
tr|F0P548|F0P548_STAPE	Holo-[acyl-carrier-protein] synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=acpS PE=3 SV=1	13,4	7	2	123.7 (M:123.7)	3	20,7	5,85

tr|F0P6Y2|F0P6Y2_STAPE	Homoserine dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=hom PE=3 SV=1	46,5	4,9	2	115.7 (M:115.7)	4	7	5,84
sp|Q5HHQ8|HPRK_STAAC	HPr kinase/phosphorylase OS=Staphylococcus aureus (strain COL) GN=hprK PE=3 SV=1	34,5	6	2	70.9 (M:70.9)	2	5,8	3,57
tr|F0P8V5|F0P8V5_STAPE	HTH-type transcriptional regulator MgrA OS=Staphylococcus pseudintermedius (strain ED99) GN=mgrA PE=4 SV=1	16,8	5,8	1	335.2 (M:335.2)	6	52,4	144,82
tr|F0P5W3|F0P5W3_STAPE	HTH-type transcriptional regulator TcaR OS=Staphylococcus pseudintermedius (strain ED99) GN=tcaR PE=4 SV=1	17,5	8	1	78.1 (M:78.1)	2	11,7	2,65
tr|F0P588|F0P588_STAPE	Hydrolase, carbon-nitrogen family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0797 PE=4 SV=1	30,3	4,9	1	226.8 (M:226.8)	5	21,7	288,11
tr|F0P9J7|F0P9J7_STAPE	Hydrolase, TatD family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2320 PE=4 SV=1	29	5,4	2	140.3 (M:140.3)	4	16,9	3,13
tr|F0P918|F0P918_STAPE	Hypoxanthine phosphoribosyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=hpt PE=4 SV=1	20,2	4,9	2	307.0 (M:307.0)	5	31,3	1,31
tr|E8SHU1|E8SHU1_STAPH	IgG-binding protein SBI OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0032 PE=4 SV=1	56,5	5,4	2	467.7 (M:467.7)	10	20,1	3,66
tr|E8SF10|E8SF10_STAPH	Imidazolonepropionase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=hutI PE=3 SV=1	45,6	5,2	2	167.8 (M:167.8)	4	11,4	3,85
tr|F0P9M2|F0P9M2_STAPE	Immunodominant staphylococcal antigen B, putative OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2350 PE=4 SV=1	19	8,6	1	512.7 (M:512.7)	8	36,3	3,25
tr|F0P5B5|F0P5B5_STAPE	inorganic pyrophosphatase	34	4,6	1	493.4 (M:493.4)	7	31,6	1,82
tr|F0P972|F0P972_STAPE	Inosine-5'-monophosphate dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=guaB PE=3 SV=1	52,4	5,3	3	1909.5 (M:1909.5)	33	70,7	4,83
tr|F0P3U2|F0P3U2_STAPE	Inositol monophosphatase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1685 PE=4 SV=1	30,2	5,2	1	130.9 (M:130.9)	2	11,8	311,02
tr|F0P658|F0P658_STAPE	Intracellular protease, PfpI family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0878 PE=4 SV=1	18,5	4,5	2	916.2 (M:916.2)	11	55,3	157,86
tr|F0P876|F0P876_STAPE	Iron compound ABC transporter periplasmic component OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2150 PE=4 SV=1	32,8	6,4	2	176.3 (M:176.3)	3	11,9	2,74
tr|F0P9G9|F0P9G9_STAPE	Iron compound ABC transporter, iron compound-binding protein OS=Staphylococcus pseudintermedius (strain ED99) GN=sirA PE=4 SV=1	36,2	6,1	3	549.8 (M:549.8)	11	35,5	4,3
tr|E8SDS7|E8SDS7_STAPH	Iron-sulfur cluster assembly protein SufD OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0537 PE=4 SV=1	48,4	4,9	3	459.3 (M:459.3)	10	27,1	4,58
tr|E8SJR5|E8SJR5_STAPH	Iron-sulfur cluster-binding protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1604 PE=4 SV=1	41,9	6,4	3	90.2 (M:90.2)	3	7,4	2,92
tr|E8SEG2|E8SEG2_STAPH	Isochorismatase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1875 PE=4 SV=1	20,3	5,2	1	71.5 (M:71.5)	1	10,5	0,5
tr|F0P3L0|F0P3L0_STAPE	Isocitrate dehydrogenase [NADP] OS=Staphylococcus pseudintermedius (strain ED99) GN=icd PE=3 SV=1	46,3	5	2	562.8 (M:562.8)	12	30,5	3,81
tr|F0P7M4|F0P7M4_STAPE	Isoleucine--tRNA ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=ileS PE=3 SV=1	104,3	5,2	6	322.5 (M:322.5)	8	7,3	6,21
tr|E8SG76|E8SG76_STAPH	Isoprenyl transferase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0986 PE=3 SV=1	29,4	6,6	3	120.5 (M:120.5)	3	15,8	292,05

tr|E8SE98|E8SE98_STAPH	kinase-associated protein b	14,6	5,8	2	80.1 (M:80.1)	2	13,5	8,85
tr|F0P5T4|F0P5T4_STAPE	lactate dehydrogenase	35,3	5,4	1	415.8 (M:415.8)	10	30,5	152,02
tr|F0P4Y9|F0P4Y9_STAPE	Leucine dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=dhlE PE=3 SV=1	39,4	5	2	401.2 (M:401.2)	7	19,5	3,54
tr|F0P765|F0P765_STAPE	Leucine--tRNA ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=leuS PE=3 SV=1	92,2	5	3	600.4 (M:600.4)	15	13,2	6,15
tr|F0P6X2|F0P6X2_STAPE	LexA repressor OS=Staphylococcus pseudintermedius (strain ED99) GN=lexA PE=3 SV=1	23,3	5,2	1	68.8 (M:68.8)	2	13	298,65
tr|E8SI63|E8SI63_STAPH	Lipase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0072 PE=4 SV=1	75,8	7,8	2	144.1 (M:144.1)	4	7,7	6,11
tr|E8SFE0|E8SFE0_STAPH	Lipoprotein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2041 PE=4 SV=1	25,7	5,4	2	348.8 (M:348.8)	5	27,4	207,23
tr|F0P807|F0P807_STAPE	Lipoyl synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=lipA PE=3 SV=1	34,8	8,6	2	132.3 (M:132.3)	4	15,7	4,8
tr|F0P760|F0P760_STAPE	L-lactate dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=ldh PE=3 SV=1	33,9	5,1	1	1195.1 (M:1195.1)	16	45,6	1,44
tr|F0P652|F0P652_STAPE	Low molecular weight protein-tyrosine-phosphatase PtpA OS=Staphylococcus pseudintermedius (strain ED99) GN=ptpA PE=4 SV=1	17,5	4,7	1	108.8 (M:108.8)	2	15,1	2,4
tr|F0P4C1|F0P4C1_STAPE	Low molecular weight protein-tyrosine-phosphatase PtpB OS=Staphylococcus pseudintermedius (strain ED99) GN=ptpB PE=4 SV=1	15,9	4,7	1	100.1 (M:100.1)	2	15,6	434,8
tr|E8SIQ4|E8SIQ4_STAPH	Lysine--tRNA ligase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=lysS PE=3 SV=1	56,8	5,3	3	350.3 (M:350.3)	9	15,4	3,85
tr|F0P380|F0P380_STAPE	lysophospholipase	31,9	4,5	1	227.6 (M:227.6)	5	25,7	2,17
tr|F0P8U9|F0P8U9_STAPE	Malate dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=mdh PE=3 SV=1	33,6	5,2	1	91.4 (M:91.4)	2	8	2,82
tr|F0P5V6|F0P5V6_STAPE	malate:quinone oxidoreductase	55,6	7,7	1	89.2 (M:89.2)	3	6	4,77
tr|F0P7I7|F0P7I7_STAPE	Malonyl CoA-acyl carrier protein transacylase OS=Staphylococcus pseudintermedius (strain ED99) GN=fabD PE=3 SV=1	33,8	4,8	1	251.1 (M:251.1)	5	24	3,48
tr|F0P9H5|F0P9H5_STAPE	manganese abc transporter substrate-binding protein	34,5	6,1	2	980.1 (M:980.1)	17	49	154,21
tr|F0P5Q4|F0P5Q4_STAPE	Metalloregulation DNA-binding stress protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0144 PE=3 SV=1	16,9	4,6	1	476.8 (M:476.8)	8	56,1	3,05
tr|F0P644|F0P644_STAPE	Methionine aminopeptidase OS=Staphylococcus pseudintermedius (strain ED99) GN=map PE=3 SV=1	27,6	5,2	1	475.2 (M:475.2)	11	42,3	185,12
tr|F0P9J8|F0P9J8_STAPE	Methionine--tRNA ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=metG PE=3 SV=1	74,8	5,2	2	704.9 (M:704.9)	15	19,8	5,54
tr|F0P7K4|F0P7K4_STAPE	Methionyl-tRNA formyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=fmt PE=3 SV=1	34	5,3	2	113.5 (M:113.5)	4	9,7	2,69
tr|E8SG67|E8SG67_STAPH	Methylenetetrahydrofolate--tRNA-(uracil-5-)-methyltransferase TrmFO OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=trmFO PE=3 SV=1	48,2	6,1	3	313.4 (M:313.4)	6	18	4,68

tr|F0P5Z2|F0P5Z2_STAPE	molybdenum cofactor biosysynthesis protein	25,3	6,8	2	159.7 (M:159.7)	2	15,5	311,44
tr|F0P6K0|F0P6K0_STAPE	Molybdopterin converting factor, subunit 2 OS=Staphylococcus pseudintermedius (strain ED99) GN=moaE PE=4 SV=1	17,3	5,8	1	103.2 (M:103.2)	2	13,8	2,71
tr|F0P660|F0P660_STAPE	monofunctional glycosyltransferase	30,8	9,5	1	162.1 (M:162.1)	4	20,4	2,64
tr|F0P551|F0P551_STAPE	mRNA interferase OS=Staphylococcus pseudintermedius (strain ED99) GN=mazF PE=3 SV=1	13,1	9,8	2	162.9 (M:162.9)	3	17,9	595,58
tr|F0P390|F0P390_STAPE	MutT/nudix family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0234 PE=4 SV=1	14,9	4,7	1	97.6 (M:97.6)	2	22,3	4,16
tr|F0P5F4|F0P5F4_STAPE	N utilization substance protein B homolog OS=Staphylococcus pseudintermedius (strain ED99) GN=nusB PE=3 SV=1	15,2	5,6	1	113.2 (M:113.2)	3	24,6	342,72
tr|F0P3Y6|F0P3Y6_STAPE	N5-carboxyaminoimidazole ribonucleotide mutase OS=Staphylococcus pseudintermedius (strain ED99) GN=purE PE=3 SV=1	17	5,5	2	113.6 (M:113.6)	3	23,1	5,22
tr|E8SH51|E8SH51_STAPH	n6-adenine-specific dna methylase	42,1	5,2	3	116.5 (M:116.5)	3	8	749,37
tr|E8SE38|E8SE38_STAPH	N-acetylglucosamine-1-phosphate uridyltransferase eukaryotic OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1833 PE=4 SV=1	45,4	5,2	2	119.1 (M:119.1)	3	7,1	4,94
tr|E8SEW4|E8SEW4_STAPH	N-acetylmannosamine kinase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1947 PE=4 SV=1	32,3	5	2	153.6 (M:153.6)	3	12,2	1,33
tr|F0P9M4|F0P9M4_STAPE	N-acetylmuramoyl-L-alanine amidase Sle1 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2352 PE=4 SV=1	36,7	9,8	1	514.1 (M:514.1)	7	29	2,98
tr|F0P859|F0P859_STAPE	NAD dependent epimerase/dehydratase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1965 PE=4 SV=1	34,3	9,3	3	271.9 (M:271.9)	6	19,7	2,37
tr|F0P8W3|F0P8W3_STAPE	NAD dependent epimerase/dehydratase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2233 PE=4 SV=1	35,7	4,7	2	75.9 (M:75.9)	2	6,9	3,94
tr|F0P8H2|F0P8H2_STAPE	nad(+) nadh kinase	30,3	6,8	2	57.9 (M:57.9)	2	7,4	3,96
tr|F0P6H0|F0P6H0_STAPE	NAD/NADP octopine/nopaline dehydrogenase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0521 PE=4 SV=1	40,3	5,1	2	242.4 (M:242.4)	7	24,6	176,01
tr|F0P3K4|F0P3K4_STAPE	NAD-dependent malic enzyme OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1100 PE=3 SV=1	44,5	5,1	2	558.0 (M:558.0)	12	41	147,4
tr|F0P7Z6|F0P7Z6_STAPE	nadh dehydrogenase	44,1	6,2	1	495.1 (M:495.1)	10	23,5	4,36
tr|F0P3T9|F0P3T9_STAPE	NADH-dependent flavin oxidoreductase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1682 PE=4 SV=1	42,1	5,1	2	725.5 (M:725.5)	14	42,4	1,88
tr|F0P4U6|F0P4U6_STAPE	nadph:quinone oxidoreductase	20,5	6,1	1	730.1 (M:730.1)	9	74,6	1,67
sp|Q5HHU4|QUEF_STAAC	NADPH-dependent 7-cyano-7-deazaguanine reductase OS=Staphylococcus aureus (strain COL) GN=queF PE=3 SV=1	19,6	5,3	3	69.7 (M:69.7)	2	14,5	380,23
tr|F0P7U9|F0P7U9_STAPE	Naphthoate synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=menB PE=4 SV=1	30,3	5,4	2	614.4 (M:614.4)	10	44,1	2,15
tr|F0P5C1|F0P5C1_STAPE	NH(3)-dependent NAD(+) synthetase OS=Staphylococcus pseudintermedius (strain ED99) GN=nadE PE=3 SV=1	30,8	5,4	2	477.7 (M:477.7)	9	36,7	4,36

tr|F0P5C0|F0P5C0_STAPE	Nicotinate phosphoribosyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=pncB PE=3 SV=1	56,3	6	1	321.5 (M:321.5)	6	14,9	238,64
tr|F0P534|F0P534_STAPE	Nitrate reductase, beta subunit OS=Staphylococcus pseudintermedius (strain ED99) GN=narH PE=4 SV=1	59,1	5,8	2	281.6 (M:281.6)	8	17,4	5,18
tr|F0P6C7|F0P6C7_STAPE	Nitric-oxide reductase, putative OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1377 PE=4 SV=1	29,5	4,7	1	125.2 (M:125.2)	3	18,1	274,91
tr|F0P523|F0P523_STAPE	Nitrite reductase [NAD(P)H], large subunit OS=Staphylococcus pseudintermedius (strain ED99) GN=nirB PE=4 SV=1	89	5,3	3	724.7 (M:724.7)	15	19,1	4,55
tr|F0P524|F0P524_STAPE	Nitrite reductase [NAD(P)H], small subunit OS=Staphylococcus pseudintermedius (strain ED99) GN=nirD PE=4 SV=1	11,4	4,5	1	203.9 (M:203.9)	3	28,8	1,07
tr|F0P801|F0P801_STAPE	Nitrogen fixation protein NifU OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1903 PE=4 SV=1	8,6	4,2	1	73.9 (M:73.9)	2	42,5	2,4
tr|F0P461|F0P461_STAPE	Nitroreductase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0318 PE=4 SV=1	23,7	4,8	2	477.9 (M:477.9)	10	47,4	2,33
tr|F0P7R0|F0P7R0_STAPE	Non-canonical purine NTP pyrophosphatase OS=Staphylococcus pseudintermedius (strain ED99) GN=rdgB PE=3 SV=1	21,2	4,5	1	50.9 (M:50.9)	2	10,3	4,69
tr|F0P9L0|F0P9L0_STAPE	Nucleoid-associated protein SPSE_2336 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2336 PE=3 SV=1	11,6	5,2	1	127.6 (M:127.6)	2	15,1	6,35
tr|F0P678|F0P678_STAPE	Nucleoside diphosphate kinase OS=Staphylococcus pseudintermedius (strain ED99) GN=ndk PE=3 SV=1	16,5	5,4	2	333.5 (M:333.5)	7	48,7	3,56
tr|F0P923|F0P923_STAPE	nucleotide pyrophosphohydrolase	45,1	4,6	1	117.1 (M:117.1)	2	5,8	1,59
tr|E8SHK3|E8SHK3_STAPH	Octaprenyl-diphosphate synthase / Dimethylallyltransferase / Geranyltranstransferase (Farnesyldiphosphate synthase) / Geranylgeranyl pyrophosphate synthetase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1218 PE=3 SV=1	32,6	5,4	2	115.3 (M:115.3)	2	8,5	5,29
tr|F0P6E7|F0P6E7_STAPE	oligoendopeptidase f	68,6	4,7	2	484.8 (M:484.8)	11	20,7	5,36
tr|F0P8H9|F0P8H9_STAPE	Oligoendopeptidase F OS=Staphylococcus pseudintermedius (strain ED99) GN=pepF PE=4 SV=1	69,6	5	3	1201.6 (M:1201.6)	22	33,9	3,95
tr|F0P8I6|F0P8I6_STAPE	Oligopeptide ABC transporter, periplasmic oligopeptide-binding protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1850 PE=4 SV=1	62	8,6	2	525.3 (M:525.3)	12	25,5	130,24
tr|E8SEL6|E8SEL6_STAPH	Oligopeptide transport ATP-binding protein OppF OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0671 PE=3 SV=1	35,6	8,7	3	99.1 (M:99.1)	3	10,5	5,73
tr|F0P8I8|F0P8I8_STAPE	Oligopeptide transport ATP-binding protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1852 PE=3 SV=1	40	5,8	1	118.5 (M:118.5)	3	7,2	2,31
tr|B1GVI3|B1GVI3_9STAP	Open reading frame X OS=Staphylococcus pseudintermedius PE=3 SV=1	12,9	9,3	4	35.6 (M:35.6)	1	12,4	7,34
tr|F0P834|F0P834_STAPE	Organic hydroperoxide resistance protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1939 PE=4 SV=1	15,5	4,6	1	635.6 (M:635.6)	9	46,4	3,27
tr|E8SEA7|E8SEA7_STAPH	Ornithine aminotransferase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=rocD PE=3 SV=1	43,7	5,2	3	102.9 (M:102.9)	2	7	6,48
tr|F0P7A1|F0P7A1_STAPE	Ornithine cyclodeaminase/mu-crystallin family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1071 PE=4 SV=1	34,9	4,8	2	218.2 (M:218.2)	5	14,5	3,74
tr|F0P7L3|F0P7L3_STAPE	Orotidine 5'-phosphate decarboxylase OS=Staphylococcus pseudintermedius (strain ED99) GN=pyrF PE=3 SV=1	25,8	5,4	2	84.8 (M:84.8)	3	10,7	5,08

tr|E8SIH5|E8SIH5_STAPH	Oxidoreductase of aldo/keto reductase family, subgroup 1 OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1390 PE=4 SV=1	31,7	5,7	2	563.5 (M:563.5)	12	42,4	144,41
tr|E8SJC7|E8SJC7_STAPH	Oxidoreductase of aldo/keto reductase family, subgroup 1 OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1548 PE=4 SV=1	31,8	5,1	3	622.8 (M:622.8)	12	39,9	2,23
tr|F0P6H4|F0P6H4_STAPE	Oxidoreductase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0525 PE=4 SV=1	38,6	5,5	1	287.0 (M:287.0)	5	22	2,68
tr|F0P470|F0P470_STAPE	Oxidoreductase, short chain dehydrogenase/reductase family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0327 PE=4 SV=1	31,4	4,6	1	458.8 (M:458.8)	9	44,7	3,48
tr|F0P397|F0P397_STAPE	Oxygen-dependent choline dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=betA PE=3 SV=1	62,9	6,9	3	616.6 (M:616.6)	15	33,3	93,85
tr|E8SI88|E8SI88_STAPH	Pantothenate synthetase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=panC PE=3 SV=1	32,2	5	2	168.0 (M:168.0)	4	14,7	4,51
tr|F0P9E9|F0P9E9_STAPE	Penicillin binding protein 4 OS=Staphylococcus pseudintermedius (strain ED99) GN=pbp4 PE=3 SV=1	47,9	9,1	2	196.3 (M:196.3)	5	13,9	224,45
tr|F0P695|F0P695_STAPE	Penicillin-binding protein 2 OS=Staphylococcus pseudintermedius (strain ED99) GN=pbp2 PE=4 SV=1	82,2	6	2	1445.4 (M:1445.4)	27	39,3	4,91
tr|F0P4N9|F0P4N9_STAPE	Penicillin-binding protein 3 OS=Staphylococcus pseudintermedius (strain ED99) GN=pbp3 PE=4 SV=1	76,3	8,3	3	62.0 (M:62.0)	2	3,7	3,68
tr|E8SKB8|E8SKB8_STAPH	Peptidase T OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=pepT PE=3 SV=1	45,4	4,8	2	286.2 (M:286.2)	6	17,7	3,37
tr|F0P5G9|F0P5G9_STAPE	Peptidase T-like protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1288 PE=4 SV=1	40,3	5,1	1	50.8 (M:50.8)	2	6,7	8,42
tr|F0P4H9|F0P4H9_STAPE	Peptidase, U32 family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1180 PE=4 SV=1	35,9	4,7	2	136.7 (M:136.7)	3	12,3	4,94
tr|E8SDY5|E8SDY5_STAPH	Peptide chain release factor 1 OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=prfA PE=3 SV=1	40,5	4,8	3	234.3 (M:234.3)	6	17,3	3,54
tr|F0P8N6|F0P8N6_STAPE	Peptide chain release factor 2 OS=Staphylococcus pseudintermedius (strain ED99) GN=prfB PE=3 SV=1	37,6	4,8	2	198.0 (M:198.0)	5	16,4	4,59
tr|F0P8F3|F0P8F3_STAPE	Peptide chain release factor 3 OS=Staphylococcus pseudintermedius (strain ED99) GN=prfC PE=3 SV=1	59,4	5,1	3	249.1 (M:249.1)	7	13,5	4,94
tr|F0P3V7|F0P3V7_STAPE	Peptide deformylase OS=Staphylococcus pseudintermedius (strain ED99) GN=defB PE=3 SV=1	20,8	5,3	1	502.9 (M:502.9)	9	34,4	2,6
tr|F0P6B2|F0P6B2_STAPE	Peptide methionine sulfoxide reductase MsrA OS=Staphylococcus pseudintermedius (strain ED99) GN=msrA1 PE=3 SV=1	19,9	4,9	2	263.5 (M:263.5)	6	27	5,14
tr|F0P6B3|F0P6B3_STAPE	Peptide methionine sulfoxide reductase MsrB OS=Staphylococcus pseudintermedius (strain ED99) GN=msrB PE=3 SV=1	16,2	4,9	1	400.3 (M:400.3)	5	43,7	1,52
tr|F0P6U8|F0P6U8_STAPE	Peptide methionine sulfoxide reductase regulator MsrR OS=Staphylococcus pseudintermedius (strain ED99) GN=msrR PE=4 SV=1	36	9,7	2	392.3 (M:392.3)	10	34,4	3,87
tr|F0P8M2|F0P8M2_STAPE	Peptidyl-prolyl cis-trans isomerase, cyclophilin-type OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1886 PE=3 SV=1	21,8	4,4	2	547.9 (M:547.9)	11	53,8	218,66
tr|E8SFJ6|E8SFJ6_STAPH	Phenylalanine--tRNA ligase alpha subunit OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=pheS PE=3 SV=1	40,2	5,3	3	177.3 (M:177.3)	5	13,2	353,79
tr|E8SFJ7|E8SFJ7_STAPH	Phenylalanine--tRNA ligase beta subunit OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=pheT PE=3 SV=1	88,5	4,9	2	379.2 (M:379.2)	7	11	3,89

tr|F0P622|F0P622_STAPE	Pheromone lipoprotein CamS OS=Staphylococcus pseudintermedius (strain ED99) GN=camS PE=4 SV=1	45,8	5,2	1	98.9 (M:98.9)	3	7,7	319,02
tr|F0P7I8|F0P7I8_STAPE	Phosphate acyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=plsX PE=3 SV=1	35,4	5,7	3	440.5 (M:440.5)	10	27,4	4,93
tr|F0P4L7|F0P4L7_STAPE	Phosphate starvation-inducible protein PhoH OS=Staphylococcus pseudintermedius (strain ED99) GN=phoH PE=4 SV=1	34,5	5,7	2	161.8 (M:161.8)	3	12,4	3,57
tr|E8SF65|E8SF65_STAPH	Phosphocarrier protein of PTS system OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0789 PE=3 SV=1	9,5	4,7	3	244.5 (M:244.5)	4	43,2	332,33
tr|F0P7Q9|F0P7Q9_STAPE	phosphodiesterase	19,3	5,3	1	100.0 (M:100.0)	2	17,8	328,61
tr|E8SF66|E8SF66_STAPH	Phosphoenolpyruvate-protein phosphotransferase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0790 PE=3 SV=1	63,5	4,7	3	527.5 (M:527.5)	12	23	5,54
tr|E8SHP6|E8SHP6_STAPH	Phosphoesterase, DHH family protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2438 PE=4 SV=1	74,4	5,2	3	137.3 (M:137.3)	4	6,6	5,66
tr|F0P7U0|F0P7U0_STAPE	Phosphoglucomutase OS=Staphylococcus pseudintermedius (strain ED99) GN=pgcA PE=3 SV=1	60,6	4,8	2	669.3 (M:669.3)	14	37,8	68,8
tr|F0P3H5|F0P3H5_STAPE	Phosphoglucosamine mutase OS=Staphylococcus pseudintermedius (strain ED99) GN=glmM PE=3 SV=1	48,8	4,8	2	602.8 (M:602.8)	14	33,9	5,52
tr|E8SJS2|E8SJS2_STAPH	Phosphoglycerate dehydrogenase-like protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1611 PE=4 SV=1	36,3	5,8	2	375.8 (M:375.8)	8	27,8	2,82
tr|F0P854|F0P854_STAPE	Phosphoglycerate kinase OS=Staphylococcus pseudintermedius (strain ED99) GN=pgk PE=3 SV=1	42,6	4,7	2	968.9 (M:968.9)	20	47,2	4,48
tr|F0P9S8|F0P9S8_STAPE	Phospholipase/carboxylesterase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2494 PE=4 SV=1	21,8	4,8	1	274.6 (M:274.6)	6	33,3	4,17
tr|F0P8B8|F0P8B8_STAPE	phosphomethylpyrimidine kinase	29,6	5	2	301.1 (M:301.1)	8	34,4	3,46
tr|F0P3S2|F0P3S2_STAPE	Phosphopantetheine adenylyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=coaD PE=3 SV=1	18,5	5,6	2	158.9 (M:158.9)	4	22,1	410,35
tr|F0P7K7|F0P7K7_STAPE	Phosphopantothenoylcysteine decarboxylase/phosphopantothenate--cysteine ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=coaBC PE=4 SV=1	44,3	6,5	1	274.6 (M:274.6)	5	14,7	3,45
tr|F0P3A9|F0P3A9_STAPE	Phosphopentomutase OS=Staphylococcus pseudintermedius (strain ED99) GN=deoB PE=3 SV=1	43,7	4,8	2	483.2 (M:483.2)	11	33,2	3,72
tr|E8SF57|E8SF57_STAPH	Phosphoribosylamine--glycine ligase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=purD PE=3 SV=1	45,2	4,8	3	399.9 (M:399.9)	7	18	3,4
tr|F0P3Y5|F0P3Y5_STAPE	Phosphoribosylaminoimidazole carboxylase, ATPase subunit OS=Staphylococcus pseudintermedius (strain ED99) GN=purK PE=4 SV=1	42,7	5,3	2	155.5 (M:155.5)	2	6,4	3,02
tr|F0P3Y4|F0P3Y4_STAPE	Phosphoribosylaminoimidazole-succinocarboxamide synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=purC PE=3 SV=1	26,4	4,7	1	363.1 (M:363.1)	8	35,2	4,06
tr|F0P3X9|F0P3X9_STAPE	Phosphoribosylformylglycinamidine cyclo-ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=purM PE=3 SV=1	36,9	4,6	2	109.5 (M:109.5)	2	8,7	0,99
tr|F0P3Y2|F0P3Y2_STAPE	Phosphoribosylformylglycinamidine synthase 1 OS=Staphylococcus pseudintermedius (strain ED99) GN=purQ PE=3 SV=1	24,4	4,9	1	303.4 (M:303.4)	7	26,9	2,89
tr|F0P3Y1|F0P3Y1_STAPE	Phosphoribosylformylglycinamidine synthase 2 OS=Staphylococcus pseudintermedius (strain ED99) GN=purL PE=3 SV=1	79,1	4,8	2	731.6 (M:731.6)	17	24,7	5,3

tr|F0P3Y3|F0P3Y3_STAPE	Phosphoribosylformylglycinamidine synthase, PurS protein OS=Staphylococcus pseudintermedius (strain ED99) GN=purS PE=4 SV=1	9,8	4,6	2	157.7 (M:157.7)	2	31,4	0,84
tr|E8SF55|E8SF55_STAPH	Phosphoribosylglycinamide formyltransferase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0779 PE=4 SV=1	21,1	5,4	2	120.5 (M:120.5)	2	14,9	495,27
tr|F0P4M5|F0P4M5_STAPE	phosphotransferase	31,1	5,6	1	213.9 (M:213.9)	6	25,8	5,9
tr|F0P7C3|F0P7C3_STAPE	Polyribonucleotide nucleotidyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=pnp PE=3 SV=1	76,9	5	2	1549.8 (M:1549.8)	32	42,6	5,29
tr|F0P3N4|F0P3N4_STAPE	Porphobilinogen deaminase OS=Staphylococcus pseudintermedius (strain ED99) GN=hemC PE=3 SV=1	34,2	4,9	3	104.2 (M:104.2)	3	9,1	452,09
tr|F0P3W3|F0P3W3_STAPE	Potassium uptake protein TrkA OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1706 PE=4 SV=1	24,2	5	2	100.5 (M:100.5)	2	12,3	4,92
tr|E8SK61|E8SK61_STAPH	Prephenate dehydratase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1668 PE=4 SV=1	30	5	2	98.4 (M:98.4)	3	10,1	4,62
tr|F0P3Q5|F0P3Q5_STAPE	Preprotein translocase, YajC subunit OS=Staphylococcus pseudintermedius (strain ED99) GN=yajC PE=4 SV=1	9,7	10	1	325.9 (M:325.9)	6	51,7	5,86
tr|F0P5E9|F0P5E9_STAPE	Proline dipeptidase OS=Staphylococcus pseudintermedius (strain ED99) GN=pepP PE=3 SV=1	39,3	5,3	1	601.2 (M:601.2)	12	35,1	3,51
tr|E8SG80|E8SG80_STAPH	Proline--tRNA ligase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=proS PE=3 SV=1	63,9	5,1	3	818.3 (M:818.3)	16	30,3	3,81
tr|F0P6S5|F0P6S5_STAPE	Protease production regulatory protein Hpr OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0965 PE=4 SV=1	20,6	6,2	1	159.0 (M:159.0)	4	18,8	5,35
tr|E8SID5|E8SID5_STAPH	Protein GrpE OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=grpE PE=3 SV=1	24,6	4,4	3	378.7 (M:378.7)	7	29,6	1,43
tr|F0P7P8|F0P7P8_STAPE	Protein MraZ OS=Staphylococcus pseudintermedius (strain ED99) GN=mraZ PE=3 SV=1	17,1	4,8	2	58.2 (M:58.2)	2	7,7	3,6
tr|F0P7B1|F0P7B1_STAPE	Protein RecA OS=Staphylococcus pseudintermedius (strain ED99) GN=recA PE=3 SV=1	37,9	5,3	2	569.6 (M:569.6)	12	28,5	199,51
tr|F0P8N7|F0P8N7_STAPE	Protein translocase subunit SecA OS=Staphylococcus pseudintermedius (strain ED99) GN=secA PE=3 SV=1	96,5	5,5	2	182.0 (M:182.0)	4	6,2	3,1
tr|F0P3Z3|F0P3Z3_STAPE	protein-disulfide isomerase	26,2	5,7	2	644.2 (M:644.2)	14	51,5	160,41
tr|E8SJN5|E8SJN5_STAPH	Protoporphyrinogen IX oxidase, aerobic OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1574 PE=4 SV=1	52,3	5,7	2	105.4 (M:105.4)	3	10,7	291,33
tr|F0P5I6|F0P5I6_STAPE	Pseudouridine synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=rluB PE=3 SV=1	28,2	9,5	1	78.2 (M:78.2)	2	8,5	0,73
tr|F0P6B4|F0P6B4_STAPE	PTS system glucose-specific IIA component OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1364 PE=4 SV=1	18	4,6	2	439.0 (M:439.0)	8	54,8	2,04
tr|F0P5L7|F0P5L7_STAPE	PTS system, mannose/fructose/N-acetylgalactosamine-specific component IIA OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0107 PE=4 SV=1	17,6	6,6	1	308.2 (M:308.2)	7	54,1	189,18
tr|F0P9J2|F0P9J2_STAPE	Pur operon repressor OS=Staphylococcus pseudintermedius (strain ED99) GN=purR PE=4 SV=1	30,4	8,5	2	187.7 (M:187.7)	4	19,4	3,26
tr|E8SE11|E8SE11_STAPH	Purine nucleoside phosphorylase DeoD-type OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=deoD PE=3 SV=1	26	4,7	3	156.5 (M:156.5)	3	17,8	238,41

tr|F0P466|F0P466_STAPE	Putative esterase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0323 PE=4 SV=1	33	5,2	1	62.1 (M:62.1)	2	6,9	1,89
tr|F0P7T0|F0P7T0_STAPE	Putative lipoprotein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1747 PE=4 SV=1	23,9	4,4	1	485.2 (M:485.2)	9	28,4	158,42
tr|F0P674|F0P674_STAPE	Putative peroxiredoxin OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0894 PE=4 SV=1	17,4	5,1	2	261.0 (M:261.0)	7	35,5	6,2
tr|F0P4B2|F0P4B2_STAPE	Putative transcriptional regulator OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0702 PE=4 SV=1	12,4	5,7	1	88.1 (M:88.1)	2	18,3	6,38
tr|B1GVI2|B1GVI2_9STAP	Putative uncharacterized protein OS=Staphylococcus pseudintermedius PE=4 SV=1	48,6	5,5	2	76.5 (M:76.5)	2	5,7	719,98
tr|F0P7Z9|F0P7Z9_STAPE	Pyridine nucleotide-disulfide oxidoreductase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1901 PE=4 SV=1	39,7	6,1	2	271.4 (M:271.4)	5	13,6	4,84
tr|F0P907|F0P907_STAPE	Pyridoxal biosynthesis lyase PdxS OS=Staphylococcus pseudintermedius (strain ED99) GN=pdxS PE=3 SV=1	31,9	5,5	2	871.8 (M:871.8)	16	49,2	2,59
tr|E8SGW9|E8SGW9_STAPH	pyridoxal-dependent decarboxylase domain protein	53,8	6,3	2	105.8 (M:105.8)	3	7,2	4,65
tr|F0P4Y0|F0P4Y0_STAPE	pyridoxamine 5 -phosphate oxidase family protein	16,4	4,6	1	283.8 (M:283.8)	4	28,9	5,89
tr|F0P492|F0P492_STAPE	Pyrimidine-nucleoside phosphorylase OS=Staphylococcus pseudintermedius (strain ED99) GN=pdp PE=4 SV=1	46,6	4,6	3	621.6 (M:621.6)	12	36	246,66
tr|E8SEY8|E8SEY8_STAPH	Pyrrolidone-carboxylate peptidase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=pcp PE=3 SV=1	23,5	5,1	2	91.1 (M:91.1)	2	10,8	2,32
tr|F0P5H6|F0P5H6_STAPE	Pyrroline-5-carboxylate reductase OS=Staphylococcus pseudintermedius (strain ED99) GN=proC PE=4 SV=1	29,9	4,8	1	122.5 (M:122.5)	3	12,2	3,95
tr|F0P3T2|F0P3T2_STAPE	Pyruvate carboxylase OS=Staphylococcus pseudintermedius (strain ED99) GN=pyc PE=3 SV=1	128,7	5,3	2	397.6 (M:397.6)	11	10,4	282,59
tr|E8SFW9|E8SFW9_STAPH	Pyruvate decarboxylase Alpha-keto-acid decarboxylase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2139 PE=3 SV=1	60,3	4,7	2	362.5 (M:362.5)	8	17,7	4,94
tr|F0P3V3|F0P3V3_STAPE	Pyruvate dehydrogenase complex E2 component, dihydrolipoamide acetyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=pdhC PE=3 SV=1	46,6	4,8	2	1494.6 (M:1494.6)	23	64,4	4,12
tr|F0P3V5|F0P3V5_STAPE	Pyruvate dehydrogenase E1 component alpha subunit OS=Staphylococcus pseudintermedius (strain ED99) GN=pdhA PE=4 SV=1	41,5	5	2	970.3 (M:970.3)	15	37	4,35
tr|F0P3V4|F0P3V4_STAPE	Pyruvate dehydrogenase E1 component subunit beta OS=Staphylococcus pseudintermedius (strain ED99) GN=pdhB PE=4 SV=1	35,4	4,8	2	1096.8 (M:1096.8)	15	55,7	3,5
tr|F0P723|F0P723_STAPE	Pyruvate ferredoxin oxidoreductase alpha chain OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1485 PE=4 SV=1	64,5	5,2	2	383.8 (M:383.8)	10	20,5	5,72
tr|F0P4Z2|F0P4Z2_STAPE	Pyruvate formate-lyase-activating enzyme OS=Staphylococcus pseudintermedius (strain ED99) GN=pflA PE=4 SV=1	28,3	5,3	1	66.3 (M:66.3)	2	12,3	3,66
tr|F0P3K8|F0P3K8_STAPE	Pyruvate kinase OS=Staphylococcus pseudintermedius (strain ED99) GN=pyk PE=3 SV=1	62,9	4,9	1	2018.1 (M:2018.1)	33	43,7	4,63
tr|E8SK18|E8SK18_STAPH	Pyruvate oxidase, CidC / Pyruvate oxidase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1625 PE=3 SV=1	64,2	5,2	2	1391.1 (M:1391.1)	25	50,8	63,52
tr|F0P9N9|F0P9N9_STAPE	Pyruvate, phosphate dikinase OS=Staphylococcus pseudintermedius (strain ED99) GN=ppdK PE=4 SV=1	96,7	4,9	2	277.5 (M:277.5)	8	10,1	5,75

tr|F0P3Q4|F0P3Q4_STAPE	Queuine tRNA-ribosyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=tgt PE=3 SV=1	43,3	5,9	2	94.5 (M:94.5)	2	5	2,73
tr|F0P7R8|F0P7R8_STAPE	Quinol oxidase subunit II OS=Staphylococcus pseudintermedius (strain ED99) GN=qoxA PE=4 SV=1	42	6,1	2	992.6 (M:992.6)	19	38,8	4,71
tr|E8SIH7|E8SIH7_STAPH	recombinase RarA	46,9	6,3	2	100.9 (M:100.9)	2	4,5	2,15
tr|F0P575|F0P575_STAPE	Redox-sensing transcriptional repressor rex OS=Staphylococcus pseudintermedius (strain ED99) GN=rex PE=3 SV=1	23,7	5,3	2	88.6 (M:88.6)	3	13	1,48
tr|F0P8I4|F0P8I4_STAPE	Regulatory protein spx OS=Staphylococcus pseudintermedius (strain ED99) GN=spxA PE=3 SV=1	15,4	5,7	2	177.6 (M:177.6)	4	33,6	5,28
tr|Q5HJZ8|Q5HJZ8_STAAC	Replication initiation protein OS=Staphylococcus aureus (strain COL) GN=repC PE=4 SV=1	37,6	6,8	3	170.3 (M:170.3)	6	12,7	4,12
tr|E8SFG7|E8SFG7_STAPH	Respiratory nitrate reductase alpha chain OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2068 PE=3 SV=1	138,5	5,8	2	150.0 (M:150.0)	4	3,4	470,45
tr|F0P5E5|F0P5E5_STAPE	Rhodanese-like domain protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1264 PE=4 SV=1	14,9	10,1	1	481.5 (M:481.5)	7	41,4	3,08
tr|F0P7C5|F0P7C5_STAPE	Riboflavin biosynthesis protein RibF OS=Staphylococcus pseudintermedius (strain ED99) GN=ribF PE=4 SV=1	36,7	5,3	2	83.3 (M:83.3)	3	12,1	4,84
tr|E8SH47|E8SH47_STAPH	Ribonuclease HI OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1144 PE=4 SV=1	14,9	5,3	2	77.8 (M:77.8)	2	15,9	416,08
tr|F0P3W1|F0P3W1_STAPE	Ribonuclease J OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1704 PE=3 SV=1	62	6,1	2	323.3 (M:323.3)	9	17,7	162,17
tr|F0P5H5|F0P5H5_STAPE	Ribonuclease Z OS=Staphylococcus pseudintermedius (strain ED99) GN=rnz PE=3 SV=1	34,3	5,9	1	210.8 (M:210.8)	5	22	297,16
tr|F0P8Q5|F0P8Q5_STAPE	Ribonucleoside-diphosphate reductase OS=Staphylococcus pseudintermedius (strain ED99) GN=nrdE PE=3 SV=1	80,2	5,5	2	516.3 (M:516.3)	11	17,5	5,66
tr|F0P8Q4|F0P8Q4_STAPE	Ribonucleoside-diphosphate reductase, beta subunit OS=Staphylococcus pseudintermedius (strain ED99) GN=nrdF PE=4 SV=1	37	4,8	2	319.5 (M:319.5)	7	23,4	4,23
tr|F0P5Z3|F0P5Z3_STAPE	Ribose-5-phosphate isomerase A OS=Staphylococcus pseudintermedius (strain ED99) GN=rpiA PE=3 SV=1	25,8	4,9	2	147.0 (M:147.0)	4	14,7	3,51
tr|F0P928|F0P928_STAPE	Ribose-phosphate pyrophosphokinase OS=Staphylococcus pseudintermedius (strain ED99) GN=prs PE=3 SV=1	35,3	5,3	2	407.5 (M:407.5)	9	36,1	157,57
tr|E8SJQ7|E8SJQ7_STAPH	Ribosomal large subunit pseudouridine synthase D, YhcT OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1596 PE=4 SV=1	31,7	6,7	3	85.6 (M:85.6)	2	7,6	1,8
tr|F0P4K9|F0P4K9_STAPE	Ribosomal protein L11 methyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=prmA PE=3 SV=1	35	4,4	1	120.0 (M:120.0)	3	13,5	410,56
tr|F0P5J7|F0P5J7_STAPE	Ribosomal protein S1 OS=Staphylococcus pseudintermedius (strain ED99) GN=rpsA PE=4 SV=1	43,5	4,6	2	1323.7 (M:1323.7)	22	62,4	3,4
tr|E8SHN5|E8SHN5_STAPH	Ribosomal RNA large subunit methyltransferase H OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=rlmH PE=3 SV=1	18,1	9,5	2	25.6 (M:25.6)	1	11,3	0,46
tr|F0P4L0|F0P4L0_STAPE	Ribosomal RNA small subunit methyltransferase E OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1211 PE=3 SV=1	28,6	6,1	1	87.6 (M:87.6)	2	8	4,41
tr|F0P7P7|F0P7P7_STAPE	Ribosomal RNA small subunit methyltransferase H OS=Staphylococcus pseudintermedius (strain ED99) GN=mraW PE=3 SV=1	35,4	6,8	1	71.6 (M:71.6)	2	8,7	3,31

tr|F0P4J6|F0P4J6_STAPE	Ribosomal silencing factor RsfS OS=Staphylococcus pseudintermedius (strain ED99) GN=rsfS PE=3 SV=1	13,5	4,9	1	124.3 (M:124.3)	3	18,6	8,25
tr|F0P8N8|F0P8N8_STAPE	Ribosomal subunit interface protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1985 PE=4 SV=1	21,7	5,3	2	415.9 (M:415.9)	8	31,2	151,74
tr|F0P7D3|F0P7D3_STAPE	Ribosome maturation factor RimP OS=Staphylococcus pseudintermedius (strain ED99) GN=rimP PE=3 SV=1	17,4	4,9	1	78.3 (M:78.3)	2	11,6	2,95
tr|F0P7C7|F0P7C7_STAPE	Ribosome-binding factor A OS=Staphylococcus pseudintermedius (strain ED99) GN=rbfA PE=3 SV=1	13,3	5,9	2	271.3 (M:271.3)	5	43	4,79
tr|F0P7E1|F0P7E1_STAPE	Ribosome-recycling factor OS=Staphylococcus pseudintermedius (strain ED99) GN=frr PE=3 SV=1	20,4	5,3	1	853.0 (M:853.0)	14	52,7	136,22
tr|F0P7J7|F0P7J7_STAPE	Ribulose-phosphate 3-epimerase OS=Staphylococcus pseudintermedius (strain ED99) GN=rpe PE=3 SV=1	23,7	4,9	1	86.2 (M:86.2)	2	12	5,97
tr|F0P4W7|F0P4W7_STAPE	ring-cleaving dioxygenase mhqO	34,2	5,5	1	421.4 (M:421.4)	9	27	4,52
tr|F0P8Y4|F0P8Y4_STAPE	RNA methyltransferase, TrmH family, group 3 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2255 PE=4 SV=1	27,1	8,9	2	320.2 (M:320.2)	8	39	4,6
tr|F0P4M7|F0P4M7_STAPE	RNA polymerase sigma factor OS=Staphylococcus pseudintermedius (strain ED99) GN=sigA PE=3 SV=1	42,3	4,9	2	341.2 (M:341.2)	8	22,8	4,9
tr|F0P922|F0P922_STAPE	RNA-binding S4 domain protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2305 PE=4 SV=1	9,5	9,8	1	76.1 (M:76.1)	2	20	6,75
tr|F0P7Y0|F0P7Y0_STAPE	rRNA adenine N-6-methyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=ermA PE=4 SV=1	28,8	9,8	2	62.9 (M:62.9)	2	6,5	2,87
tr|F0P920|F0P920_STAPE	S1 RNA binding domain protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2303 PE=4 SV=1	13,9	9,5	2	309.7 (M:309.7)	8	43,2	4,92
tr|F0P739|F0P739_STAPE	S-adenosylmethionine synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=metK PE=3 SV=1	44,1	4,9	3	387.2 (M:387.2)	7	20,6	2,78
tr|E8SII9|E8SII9_STAPH	S-adenosylmethionine:tRNA ribosyltransferase-isomerase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=queA PE=3 SV=1	38,8	5,5	2	52.4 (M:52.4)	2	5,9	433,64
tr|F0P7X7|F0P7X7_STAPE	SAM-dependent methyltransferase	28,2	5,9	1	118.3 (M:118.3)	2	9	2,7
tr|E8SEX5|E8SEX5_STAPH	Secretory antigen SsaA OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1958 PE=4 SV=1	25,5	6,5	3	808.4 (M:808.4)	7	46,3	0,34
tr|F0P3I5|F0P3I5_STAPE	Septation ring formation regulator EzrA OS=Staphylococcus pseudintermedius (strain ED99) GN=ezrA PE=4 SV=1	66,7	4,8	3	796.5 (M:796.5)	15	27,9	173,34
tr|F0P4C3|F0P4C3_STAPE	Serine hydroxymethyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=glyA PE=3 SV=1	45,3	6	3	1260.0 (M:1260.0)	25	58,7	5,54
tr|F0P6B6|F0P6B6_STAPE	serine protease	56,9	8,2	3	74.9 (M:74.9)	2	4,9	6,47
tr|E8SHQ1|E8SHQ1_STAPH	Serine--tRNA ligase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=serS PE=3 SV=1	48,4	5,1	3	668.1 (M:668.1)	14	42,7	116,52
tr|F0P4J2|F0P4J2_STAPE	Shikimate dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=aroE PE=3 SV=1	30,1	5,5	2	89.0 (M:89.0)	2	7	5,41
tr|F0P3F5|F0P3F5_STAPE	Siderophore biosynthesis protein, IucA/IucC family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0632 PE=4 SV=1	74,5	5,4	2	61.5 (M:61.5)	2	2,8	4,98

tr|F0P8K7|F0P8K7_STAPE	Signal peptidase IB OS=Staphylococcus pseudintermedius (strain ED99) GN=sipB PE=3 SV=1	21,9	6,5	2	302.0 (M:302.0)	5	29,7	3,09
tr|F0P7I0|F0P7I0_STAPE	Signal recognition particle protein OS=Staphylococcus pseudintermedius (strain ED99) GN=ffh PE=3 SV=1	50,3	8,9	3	80.3 (M:80.3)	2	5,5	3,19
tr|F0P7I2|F0P7I2_STAPE	Signal recognition particle receptor FtsY OS=Staphylococcus pseudintermedius (strain ED99) GN=ftsY PE=3 SV=1	44,7	4,6	1	172.6 (M:172.6)	5	12	308,51
tr|E8SJN8|E8SJN8_STAPH	Signal transduction protein TRAP (Target of RNAIII-activating protein) OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1577 PE=4 SV=1	19,7	5,4	2	101.2 (M:101.2)	2	11,3	3,67
tr|F0P3Q7|F0P3Q7_STAPE	Single-strand DNA-specific exonuclease RecJ OS=Staphylococcus pseudintermedius (strain ED99) GN=recJ PE=4 SV=1	85,8	5	2	60.9 (M:60.9)	2	2,8	6,77
tr|F0P995|F0P995_STAPE	Single-stranded DNA-binding protein OS=Staphylococcus pseudintermedius (strain ED99) GN=ssb PE=4 SV=1	18,7	5,3	5	435.3 (M:435.3)	7	37,5	3,1
tr|F0P4Y5|F0P4Y5_STAPE	Sortase A OS=Staphylococcus pseudintermedius (strain ED99) GN=srtA PE=4 SV=1	22,4	5,6	1	97.6 (M:97.6)	3	15,2	4,05
tr|E8SE08|E8SE08_STAPH	S-ribosylhomocysteine lyase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=luxS PE=3 SV=1	17,6	5,2	2	265.5 (M:265.5)	5	26,9	264,84
tr|F0P930|F0P930_STAPE	stage V sporulation protein G	11,8	4,6	2	366.4 (M:366.4)	7	41,3	4,27
tr|F0P9I8|F0P9I8_STAPE	Staphylococcal accessory regulator A OS=Staphylococcus pseudintermedius (strain ED99) GN=sarA PE=3 SV=1	14,7	7,8	1	368.4 (M:368.4)	7	39	1,24
tr|F0P6Y6|F0P6Y6_STAPE	Staphylococcal accessory regulator family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1447 PE=3 SV=1	14,2	7	2	307.7 (M:307.7)	7	38,3	6,21
tr|F0P7X6|F0P7X6_STAPE	Streptomycin aminoglycoside 6-adenylyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=aadE PE=4 SV=1	36,1	4,9	1	102.8 (M:102.8)	3	10,6	2,35
tr|F0P7R3|F0P7R3_STAPE	Succinate dehydrogenase flavoprotein subunit OS=Staphylococcus pseudintermedius (strain ED99) GN=sdhA PE=4 SV=1	65,3	5,6	2	697.1 (M:697.1)	16	32,1	5,5
tr|F0P7R2|F0P7R2_STAPE	Succinate dehydrogenase iron-sulfur subunit OS=Staphylococcus pseudintermedius (strain ED99) GN=sdhB PE=4 SV=1	30,5	7,5	2	754.5 (M:754.5)	15	54,6	155,96
tr|F0P406|F0P406_STAPE	Succinate-semialdehyde dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=gabD PE=3 SV=1	51,1	5,3	2	336.5 (M:336.5)	7	18,2	4,46
tr|F0P7F2|F0P7F2_STAPE	Succinyl-CoA ligase [ADP-forming] subunit alpha OS=Staphylococcus pseudintermedius (strain ED99) GN=sucD PE=3 SV=1	31,4	5,3	2	477.2 (M:477.2)	7	30,6	2,18
tr|E8SG63|E8SG63_STAPH	Succinyl-CoA ligase [ADP-forming] subunit beta OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=sucC PE=3 SV=1	42,2	4,9	3	483.1 (M:483.1)	11	34	4,27
tr|F0P4T3|F0P4T3_STAPE	succinyl-diaminopimelate desuccinylase	43,2	5	1	189.0 (M:189.0)	4	9,8	2,46
sp|Q5HFK7|SODM1_STAAC	Superoxide dismutase [Mn/Fe] 1 OS=Staphylococcus aureus (strain COL) GN=sodA PE=3 SV=1	22,7	5,1	1	81.1 (M:81.1)	2	8	0,18
tr|F0P4N8|F0P4N8_STAPE	Superoxide dismutase OS=Staphylococcus pseudintermedius (strain ED99) GN=sodA PE=3 SV=1	22,7	5,2	13	375.7 (M:375.7)	7	30,2	1,94
tr|F0P6U2|F0P6U2_STAPE	Synergohymenotropic toxin OS=Staphylococcus pseudintermedius (strain ED99) GN=lukS-I PE=4 SV=1	35,1	9,4	1	87.3 (M:87.3)	2	7,4	2,93
tr|F0P5L3|F0P5L3_STAPE	tagatose-6-phosphate ketose isomerase	41,6	5,2	1	133.9 (M:133.9)	4	14,8	4,9

tr|F0P9F6|F0P9F6_STAPE	Teichoic acids export ATP-binding protein TagH OS=Staphylococcus pseudintermedius (strain ED99) GN=tagH PE=3 SV=1	29,8	8, 9	1	69.8 (M:69.8)	3	9,8	503,52
tr|F0P6D0|F0P6D0_STAPE	Tellurite resistance protein, putative OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1382 PE=4 SV=1	44,8	5, 3	3	466.3 (M:466.3)	11	27,1	5,78
tr|F0P682|F0P682_STAPE	tetratricopeptide repeat protein	48,1	4, 1	2	261.7 (M:261.7)	6	24,7	4,94
tr|F0P4E4|F0P4E4_STAPE	Thiamine-phosphate synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=thiE PE=3 SV=1	23,6	5	1	141.9 (M:141.9)	4	21,2	3,61
tr|F0P9C2|F0P9C2_STAPE	Thiazole synthase OS=Staphylococcus pseudintermedius (strain ED99) GN=thiG PE=3 SV=1	27,4	4, 9	2	56.8 (M:56.8)	2	7,1	6,55
tr|F0P4G3|F0P4G3_STAPE	ThiF family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1164 PE=4 SV=1	28,2	7	2	128.9 (M:128.9)	2	10,2	6,67
tr|F0P365|F0P365_STAPE	ThiJ/PfpI family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0208 PE=4 SV=1	27,3	5, 9	2	138.2 (M:138.2)	3	16,3	2,51
tr|F0P6W0|F0P6W0_STAPE	Thioesterase family protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1421 PE=4 SV=1	17,9	5, 2	2	72.0 (M:72.0)	2	16,9	0,45
tr|F0P3J2|F0P3J2_STAPE	thiol peroxidase	18,3	4, 3	2	383.4 (M:383.4)	5	40,9	200,69
tr|F0P7R6|F0P7R6_STAPE	Thioredoxin OS=Staphylococcus pseudintermedius (strain ED99) GN=trxA PE=3 SV=1	11,5	4, 5	2	832.0 (M:832.0)	13	99	2,55
tr|F0P866|F0P866_STAPE	Thioredoxin reductase OS=Staphylococcus pseudintermedius (strain ED99) GN=trxB PE=3 SV=1	33,4	5	2	368.6 (M:368.6)	6	22,9	3,03
tr|F0P4R3|F0P4R3_STAPE	Threonine dehydratase, catabolic OS=Staphylococcus pseudintermedius (strain ED99) GN=ilvA-1 PE=3 SV=1	37,2	5, 2	1	1383.8 (M:1383.8)	22	60,1	3,57
tr|E8SGN2|E8SGN2_STAPH	Threonine synthase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1060 PE=3 SV=1	38,1	5	3	158.7 (M:158.7)	4	13,3	254,27
tr|F0P3M1|F0P3M1_STAPE	Threonine--tRNA ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=thrS PE=3 SV=1	74,6	5, 4	2	933.2 (M:933.2)	20	30,1	5,22
tr|F0P9K6|F0P9K6_STAPE	Thymidylate kinase OS=Staphylococcus pseudintermedius (strain ED99) GN=tmk PE=3 SV=1	22,8	5, 5	1	126.0 (M:126.0)	3	18,5	2,98
tr|E8SH42|E8SH42_STAPH	Thymidylate synthase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=thyA PE=3 SV=1	36,6	5, 5	3	141.7 (M:141.7)	4	12	5,09
tr|F0P745|F0P745_STAPE	transaldolase	25,7	4, 6	2	938.2 (M:938.2)	16	58,1	2,04
tr|F0P4I2|F0P4I2_STAPE	Transcription elongation factor GreA OS=Staphylococcus pseudintermedius (strain ED99) GN=greA PE=3 SV=1	17,8	4, 5	2	186.6 (M:186.6)	4	38	439,46
tr|F0P8T9|F0P8T9_STAPE	Transcription repressor of fructose operon OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2042 PE=4 SV=1	27,9	6, 3	1	252.6 (M:252.6)	5	25,6	208,3
tr|F0P4B5|F0P4B5_STAPE	Transcription termination factor Rho OS=Staphylococcus pseudintermedius (strain ED99) GN=rho PE=3 SV=1	49,7	8, 3	1	54.0 (M:54.0)	2	5,5	492,71
tr|E8SG84|E8SG84_STAPH	Transcription termination protein NusA OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0994 PE=4 SV=1	43,8	4, 6	3	582.5 (M:582.5)	11	30,4	237,79
tr|E8SJ15|E8SJ15_STAPH	Transcription termination/antitermination protein nusG OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0210 PE=3 SV=1	20,6	5, 1	3	255.1 (M:255.1)	5	34,6	1,64

tr|F0P5W6|F0P5W6_STAPE	Transcriptional activator, TenA/Thi-4 family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0455 PE=4 SV=1	26	4,6	2	89.0 (M:89.0)	2	9,8	4,08
tr|F0P902|F0P902_STAPE	Transcriptional regulator CtsR OS=Staphylococcus pseudintermedius (strain ED99) GN=ctsR PE=4 SV=1	17,8	6,1	2	387.2 (M:387.2)	8	51	4,49
tr|F0P6F8|F0P6F8_STAPE	Transcriptional regulator OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0507 PE=4 SV=1	34,9	6,1	2	438.0 (M:438.0)	10	44,5	3,94
tr|F0P5H3|F0P5H3_STAPE	Transcriptional regulator, AraC family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1294 PE=4 SV=1	33	4,9	1	73.6 (M:73.6)	2	6,3	491,49
tr|F0P6R6|F0P6R6_STAPE	Transcriptional regulator, Cro/CI family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0956 PE=4 SV=1	17,7	7,6	2	103.5 (M:103.5)	3	20,8	370,06
tr|F0P5I0|F0P5I0_STAPE	Transcriptional regulator, Fur family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1301 PE=4 SV=1	17,5	5,7	2	346.7 (M:346.7)	7	38,4	3,8
tr|F0P7U2|F0P7U2_STAPE	Transcriptional regulator, LytR family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1759 PE=4 SV=1	44,4	6	1	212.8 (M:212.8)	4	14,5	238,25
tr|F0P6K5|F0P6K5_STAPE	Transcriptional regulator, MarR family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0556 PE=4 SV=1	17,2	6,5	1	29.4 (M:29.4)	1	10,8	3,58
tr|F0P4G5|F0P4G5_STAPE	Transcriptional regulator, putative OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1166 PE=4 SV=1	15,5	6,6	1	473.4 (M:473.4)	8	61,9	162,54
tr|F0P5I7|F0P5I7_STAPE	Transcriptional regulatory protein SrrA OS=Staphylococcus pseudintermedius (strain ED99) GN=srrA PE=4 SV=1	27,8	5,3	2	275.7 (M:275.7)	6	25,3	3,98
tr|F0P464|F0P464_STAPE	transglycosylase	24,6	5,7	1	532.8 (M:532.8)	5	30,6	151,58
tr|F0P6W9|F0P6W9_STAPE	Transketolase OS=Staphylococcus pseudintermedius (strain ED99) GN=tkt PE=4 SV=1	72,2	5,1	4	2797.4 (M:2797.4)	44	64,8	4,88
tr|F0P3B8|F0P3B8_STAPE	Translation initiation factor IF-1 OS=Staphylococcus pseudintermedius (strain ED99) GN=infA PE=3 SV=1	8,3	6,7	1	158.4 (M:158.4)	3	47,2	1,69
tr|E8SGG9|E8SGG9_STAPH	Translation initiation factor IF-2 OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=infB PE=3 SV=1	78,1	5,2	3	176.7 (M:176.7)	4	7,9	3,99
tr|F0P3M4|F0P3M4_STAPE	Translation initiation factor IF-3 OS=Staphylococcus pseudintermedius (strain ED99) GN=infC PE=3 SV=1	20,1	9,9	2	363.2 (M:363.2)	7	41,1	2,86
tr|E8SG89|E8SG89_STAPH	Triacylglycerol lipase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2177 PE=4 SV=1	76,7	5,4	6	681.8 (M:681.8)	13	24,2	4,9
tr|F0P3M9|F0P3M9_STAPE	Trigger factor OS=Staphylococcus pseudintermedius (strain ED99) GN=tig PE=3 SV=1	49,2	4,2	1	760.2 (M:760.2)	14	21,9	136,28
tr|F0P853|F0P853_STAPE	Triosephosphate isomerase OS=Staphylococcus pseudintermedius (strain ED99) GN=tpiA PE=3 SV=1	27,3	4,9	1	924.1 (M:924.1)	15	54,2	2,88
tr|F0P6Q4|F0P6Q4_STAPE	trna (cytidine -2 -o)-methyltransferase	18,1	5,5	2	108.2 (M:108.2)	3	16,7	4,76
tr|F0P9T5|F0P9T5_STAPE	tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG OS=Staphylococcus pseudintermedius (strain ED99) GN=mnmG PE=3 SV=1	69,9	5,5	3	188.8 (M:188.8)	6	9,3	2,88
tr|F0P781|F0P781_STAPE	tRNA-binding protein	21,9	4,7	1	199.6 (M:199.6)	3	16,1	2,28
tr|F0P4H0|F0P4H0_STAPE	tRNA-specific 2-thiouridylase MnmA OS=Staphylococcus pseudintermedius (strain ED99) GN=trmU PE=3 SV=1	41,4	5	2	94.6 (M:94.6)	3	8,9	4,59

tr|E8SEL8|E8SEL8_STAPH	Tryptophan--tRNA ligase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=trpS PE=3 SV=1	37	5,8	3	265.1 (M:265.1)	6	17,3	3,48
tr|F0P539|F0P539_STAPE	Two-component response regulator NreC OS=Staphylococcus pseudintermedius (strain ED99) GN=nreC PE=4 SV=1	24,3	5,2	2	220.9 (M:220.9)	5	27,3	227,27
tr|E8SHB9|E8SHB9_STAPH	Two-component response regulator OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2393 PE=4 SV=1	22,4	5,3	2	80.0 (M:80.0)	2	11	4,97
tr|F0P4Q2|F0P4Q2_STAPE	Two-component response regulator VicR OS=Staphylococcus pseudintermedius (strain ED99) GN=vicR PE=4 SV=1	27,3	5,2	2	125.8 (M:125.8)	3	16,3	412,38
tr|F0P648|F0P648_STAPE	Two-component response regulator VraR OS=Staphylococcus pseudintermedius (strain ED99) GN=vraR PE=4 SV=1	23,5	5,3	1	161.6 (M:161.6)	3	21,5	527,27
tr|F0P3Z9|F0P3Z9_STAPE	Two-component response regulator, LytR OS=Staphylococcus pseudintermedius (strain ED99) GN=lytR PE=4 SV=1	27,9	5,4	1	90.7 (M:90.7)	3	12,7	581,37
tr|F0P793|F0P793_STAPE	Tyrosine--tRNA ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=tyrS PE=3 SV=1	47,6	5,1	2	547.7 (M:547.7)	13	31	4,28
tr|F0P6J0|F0P6J0_STAPE	UDP-glucose 4-epimerase OS=Staphylococcus pseudintermedius (strain ED99) GN=galE PE=3 SV=1	36,9	5,1	2	101.7 (M:101.7)	3	8,2	4,8
tr|E8SKB3|E8SKB3_STAPH	UDP-N-acetylenolpyruvoylglucosamine reductase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=murB PE=3 SV=1	34,3	5,2	3	382.0 (M:382.0)	8	25,2	4,42
tr|F0P4D6|F0P4D6_STAPE	UDP-N-acetylglucosamine 1-carboxyvinyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=murA PE=3 SV=1	45	5,5	2	519.5 (M:519.5)	11	30,9	4,38
tr|F0P4C5|F0P4C5_STAPE	UDP-N-acetylglucosamine 2-epimerase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0715 PE=3 SV=1	42,7	5,2	3	530.6 (M:530.6)	11	31,4	3,46
tr|F0P6B8|F0P6B8_STAPE	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase OS=Staphylococcus pseudintermedius (strain ED99) GN=murG PE=3 SV=1	40,2	6,4	2	130.2 (M:130.2)	4	15,2	231,36
tr|F0P641|F0P641_STAPE	UDP-N-acetylmuramate--alanine ligase	48,8	5,8	1	341.2 (M:341.2)	9	16,3	6,18
tr|E8SJ86|E8SJ86_STAPH	UDP-N-acetylmuramate--L-alanine ligase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=murC PE=3 SV=1	49,2	4,9	2	259.0 (M:259.0)	7	18,8	4,54
tr|E8SFP0|E8SFP0_STAPH	UDP-N-acetylmuramoylalanine--D-glutamate ligase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=murD PE=3 SV=1	49,9	6	2	332.5 (M:332.5)	7	18,5	4,26
tr|F0P8F5|F0P8F5_STAPE	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--L-lysine ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=murE PE=3 SV=1	54	5,4	3	368.0 (M:368.0)	10	25,1	4,7
tr|F0P4F6|F0P4F6_STAPE	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase OS=Staphylococcus pseudintermedius (strain ED99) GN=murF PE=3 SV=1	50	5	2	84.8 (M:84.8)	2	9,7	251,59
tr|F0P7Q0|F0P7Q0_STAPE	Uncharacterized N-acetyltransferase SPSE_1633 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1633 PE=3 SV=1	17,4	5	2	320.4 (M:320.4)	6	38,9	1,58
tr|Q5HJ42|Q5HJ42_STAAC	Uncharacterized protein OS=Staphylococcus aureus (strain COL) GN=SACOL0323 PE=4 SV=1	11,9	7,9	1	24.1 (M:24.1)	1	12,7	0,18
tr|Q5HFW4|Q5HFW4_STAAC	Uncharacterized protein OS=Staphylococcus aureus (strain COL) GN=SACOL1499 PE=4 SV=1	12,2	5,1	2	63.1 (M:63.1)	2	21	1,69
tr|F0P4W5|F0P4W5_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0088 PE=4 SV=1	18	4,7	1	63.0 (M:63.0)	2	9,3	3,85
tr|F0P4W6|F0P4W6_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0089 PE=4 SV=1	16,8	4,7	1	368.4 (M:368.4)	6	51,7	5,59

tr|F0P5M2|F0P5M2_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0112 PE=4 SV=1	18,9	5	1	326.4 (M:326.4)	4	28,1	1,47
tr|F0P5Q1|F0P5Q1_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0141 PE=4 SV=1	19,9	5,5	1	458.6 (M:458.6)	8	40,3	4,87
tr|F0P5Q3|F0P5Q3_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0143 PE=4 SV=1	12,5	4,6	1	67.5 (M:67.5)	1	11,2	1,7
tr|F0P341|F0P341_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0182 PE=4 SV=1	19,4	4,4	2	288.1 (M:288.1)	6	40,6	3,39
tr|F0P459|F0P459_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0316 PE=4 SV=1	31,8	9,5	1	133.0 (M:133.0)	3	10,6	1,89
tr|F0P4Z4|F0P4Z4_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0365 PE=4 SV=1	23,6	5,1	2	417.7 (M:417.7)	7	43,8	2,16
tr|F0P546|F0P546_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0422 PE=4 SV=1	13,9	9,3	1	171.9 (M:171.9)	4	39	4,52
tr|F0P5T7|F0P5T7_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0426 PE=4 SV=1	12,5	6,2	1	218.1 (M:218.1)	4	32,4	7,06
tr|F0P6H5|F0P6H5_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0526 PE=4 SV=1	12,5	9,4	1	265.3 (M:265.3)	6	31,6	3,56
tr|F0P3F1|F0P3F1_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0628 PE=4 SV=1	21,3	9,4	2	427.1 (M:427.1)	6	28,6	4,3
tr|F0P3F2|F0P3F2_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0629 PE=4 SV=1	9,5	9,4	2	21.9 (M:21.9)	1	12,3	6,94
tr|F0P3G3|F0P3G3_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0640 PE=4 SV=1	10,1	5,9	2	528.4 (M:528.4)	10	86	6,21
tr|F0P3H3|F0P3H3_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0659 PE=4 SV=1	29,7	6,3	2	116.9 (M:116.9)	3	12,3	5,63
tr|F0P490|F0P490_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0679 PE=4 SV=1	15,7	9	1	116.5 (M:116.5)	3	21,6	3,03
tr|F0P497|F0P497_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0687 PE=4 SV=1	26,3	4,5	1	90.0 (M:90.0)	2	13	4,71
tr|F0P5B0|F0P5B0_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0819 PE=4 SV=1	15,1	5,5	2	164.1 (M:164.1)	3	30,2	0,69
tr|F0P5B1|F0P5B1_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0820 PE=4 SV=1	6,4	5,1	1	90.7 (M:90.7)	1	22,8	4,92
tr|F0P6P8|F0P6P8_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0937 PE=4 SV=1	8,3	5,2	1	71.1 (M:71.1)	2	48,1	258,95
tr|F0P6S6|F0P6S6_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0966 PE=4 SV=1	13	9,3	2	312.8 (M:312.8)	5	45,8	6,72
tr|F0P740|F0P740_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1009 PE=4 SV=1	35,2	5,9	3	791.9 (M:791.9)	16	56,4	5,16
tr|F0P777|F0P777_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1047 PE=4 SV=1	12,2	9	2	77.8 (M:77.8)	2	22,9	0,8
tr|F0P784|F0P784_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1054 PE=4 SV=1	22,1	5,9	2	895.4 (M:895.4)	11	63,9	4,24

tr|F0P785|F0P785_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1055 PE=4 SV=1	39,3	9,1	2	923.4 (M:923.4)	14	45,5	200,26
tr|F0P4L4|F0P4L4_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1215 PE=4 SV=1	24	5,7	2	83.4 (M:83.4)	2	8,8	1,53
tr|F0P5F3|F0P5F3_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1272 PE=4 SV=1	13,7	6,4	1	120.9 (M:120.9)	2	16,1	6,82
tr|F0P5G7|F0P5G7_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1286 PE=4 SV=1	16,3	4,9	2	254.3 (M:254.3)	5	35,2	289,62
tr|F0P6A8|F0P6A8_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1358 PE=4 SV=1	16,2	4,7	1	139.2 (M:139.2)	3	23,3	4,51
tr|F0P6Y7|F0P6Y7_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1448 PE=4 SV=1	34	6	1	94.0 (M:94.0)	2	6,6	0,7
tr|F0P719|F0P719_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1481 PE=4 SV=1	14,5	5	1	37.4 (M:37.4)	1	12	0,98
tr|F0P724|F0P724_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1486 PE=4 SV=1	29,7	6,1	2	316.6 (M:316.6)	7	25	2,82
tr|F0P7J4|F0P7J4_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1577 PE=4 SV=1	13,5	5,1	2	192.2 (M:192.2)	4	37,9	5,17
tr|F0P7N7|F0P7N7_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1620 PE=3 SV=1	25,5	5,8	3	74.0 (M:74.0)	2	8	3,8
tr|F0P7N8|F0P7N8_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1621 PE=4 SV=1	30,8	4,8	2	71.3 (M:71.3)	2	8,9	5,74
tr|F0P3S4|F0P3S4_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1667 PE=4 SV=1	15,3	5,8	1	68.9 (M:68.9)	2	14	4,01
tr|F0P3S5|F0P3S5_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1668 PE=4 SV=1	9,9	9,5	1	36.1 (M:36.1)	1	14,8	6,65
tr|F0P3S7|F0P3S7_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1670 PE=4 SV=1	16,8	5,1	1	67.8 (M:67.8)	2	14,4	0,83
tr|F0P3S8|F0P3S8_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1671 PE=4 SV=1	41,2	5,2	1	48.5 (M:48.5)	2	10,1	305,64
tr|F0P3U4|F0P3U4_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1687 PE=4 SV=1	26	5,7	1	111.6 (M:111.6)	2	13,5	0,34
tr|F0P3W9|F0P3W9_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1712 PE=4 SV=1	19,7	9,9	1	206.9 (M:206.9)	4	24,4	2,97
tr|F0P805|F0P805_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1907 PE=4 SV=1	10,2	4,3	2	176.2 (M:176.2)	4	43,2	4,16
tr|F0P871|F0P871_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1978 PE=4 SV=1	13,2	5,3	1	114.2 (M:114.2)	2	13,8	4,28
tr|F0P9B1|F0P9B1_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2066 PE=4 SV=1	11,5	5,1	1	141.0 (M:141.0)	3	44,4	1,84
tr|F0P9C8|F0P9C8_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2084 PE=4 SV=1	23,9	4,7	1	48.3 (M:48.3)	2	11,7	3,27
tr|F0P9I6|F0P9I6_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2142 PE=4 SV=1	9,5	8,7	2	33.2 (M:33.2)	1	11,2	6,96

tr|F0P9J0|F0P9J0_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2146 PE=4 SV=1	18,4	9,4	1	238.8 (M:238.8)	4	29,3	6,76
tr|F0P8B6|F0P8B6_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2192 PE=4 SV=1	11,6	4,8	2	64.8 (M:64.8)	1	14	6,02
tr|F0P8E0|F0P8E0_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2218 PE=4 SV=1	13,5	5,7	2	128.3 (M:128.3)	2	17,6	1,63
tr|F0P9K5|F0P9K5_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2328 PE=4 SV=1	11,8	4,3	1	21.3 (M:21.3)	1	15,6	1,28
tr|F0P975|F0P975_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2443 PE=4 SV=1	16,2	4,9	1	723.4 (M:723.4)	13	82,1	4,84
tr|F0P986|F0P986_STAPE	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2455 PE=4 SV=1	35	5,1	2	169.3 (M:169.3)	4	23,3	230,42
tr|E8SJ37|E8SJ37_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0232 PE=4 SV=1	4,6	9	1	29.9 (M:29.9)	1	18,4	4,48
tr|E8SJW1|E8SJW1_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0380 PE=4 SV=1	18,6	5	2	97.9 (M:97.9)	3	23,3	277,44
tr|E8SE90|E8SE90_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0627 PE=4 SV=1	13,6	6,4	2	120.4 (M:120.4)	3	23,2	6,64
tr|E8SG62|E8SG62_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0972 PE=4 SV=1	10,8	4,3	2	78.1 (M:78.1)	1	15,4	2,8
tr|E8SGJ6|E8SGJ6_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1024 PE=4 SV=1	11,1	4,3	2	50.1 (M:50.1)	1	10,1	3,06
tr|E8SGP0|E8SGP0_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1068 PE=4 SV=1	31,2	6,1	2	83.0 (M:83.0)	2	9,2	4,76
tr|E8SHV0|E8SHV0_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1247 PE=4 SV=1	13,5	4,6	1	52.8 (M:52.8)	2	16,5	5,96
tr|E8SIC8|E8SIC8_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1343 PE=4 SV=1	18	5,5	2	123.7 (M:123.7)	3	20,4	357,69
tr|E8SIT4|E8SIT4_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1435 PE=4 SV=1	23,2	4,8	2	168.5 (M:168.5)	3	14,8	5,02
tr|E8SJ89|E8SJ89_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1510 PE=4 SV=1	33,5	5,8	2	123.7 (M:123.7)	4	12,2	5,44
tr|E8SJN4|E8SJN4_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1573 PE=4 SV=1	21,1	5	2	117.8 (M:117.8)	3	20,1	2,66
tr|E8SEY6|E8SEY6_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1969 PE=4 SV=1	17,3	9,4	2	147.6 (M:147.6)	3	15,5	2,8
tr|E8SG92|E8SG92_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2180 PE=4 SV=1	38,1	6,6	2	353.8 (M:353.8)	8	28,3	188,07
tr|E8SGS5|E8SGS5_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2281 PE=4 SV=1	20,1	4,8	2	59.5 (M:59.5)	2	13,5	5,1
tr|E8SH95|E8SH95_STAPH	Uncharacterized protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2369 PE=4 SV=1	16,8	4,7	1	328.8 (M:328.8)	6	55,8	5,59
tr|F0P3J5|F0P3J5_STAPE	Universal stress protein family OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1091 PE=4 SV=1	18,6	4,9	2	1010.8 (M:1010.8)	12	72,5	1,87

tr|F0P865|F0P865_STAPE	UPF0042 nucleotide-binding protein SPSE_1972 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1972 PE=3 SV=1	34,8	6	2	156.1 (M:156.1)	2	9,6	2,99
tr|F0P7I1|F0P7I1_STAPE	UPF0122 protein SPSE_1564 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1564 PE=3 SV=1	13,8	5,1	1	81.9 (M:81.9)	2	14,4	6,96
tr|F0P6X0|F0P6X0_STAPE	UPF0291 protein SPSE_1431 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1431 PE=3 SV=1	9	7,9	1	46.5 (M:46.5)	2	26,9	506,48
tr|F0P6R8|F0P6R8_STAPE	UPF0342 protein SPSE_0958 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0958 PE=3 SV=1	13,3	4,3	1	632.0 (M:632.0)	14	74,6	3,79
tr|F0P3W0|F0P3W0_STAPE	UPF0356 protein SPSE_1703 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1703 PE=3 SV=1	8,8	5,4	2	196.4 (M:196.4)	4	66,2	4,88
sp|Q5HFI7|Y1630_STAAC	UPF0365 protein SACOL1630 OS=Staphylococcus aureus (strain COL) GN=SACOL1630 PE=3 SV=1	35,2	5,5	1	369.2 (M:369.2)	6	17,3	2,88
tr|F0P4L5|F0P4L5_STAPE	UPF0365 protein SPSE_1216 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1216 PE=3 SV=1	35,2	6,1	1	860.6 (M:860.6)	17	56,2	4,68
tr|F0P8A0|F0P8A0_STAPE	UPF0447 protein SPSE_2174 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_2174 PE=3 SV=1	29,6	5,1	2	168.3 (M:168.3)	4	14,3	4,32
tr|F0P4H6|F0P4H6_STAPE	UPF0473 protein SPSE_1177 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1177 PE=3 SV=1	12,4	3,9	1	120.6 (M:120.6)	2	21,7	3,57
tr|F0P3U3|F0P3U3_STAPE	UPF0637 protein SPSE_1686 OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_1686 PE=3 SV=1	23,8	6,4	2	198.3 (M:198.3)	5	26,1	3,54
tr|F0P4C4|F0P4C4_STAPE	Uracil phosphoribosyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=upp PE=3 SV=1	23	5,4	2	744.5 (M:744.5)	14	69,4	3,32
tr|F0P8B7|F0P8B7_STAPE	Uracil-DNA glycosylase OS=Staphylococcus pseudintermedius (strain ED99) GN=ung PE=3 SV=1	25,6	6,2	1	94.4 (M:94.4)	2	11,3	5,3
tr|E8SHS5|E8SHS5_STAPH	Urease accessory protein UreE OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=ureE PE=3 SV=1	17,5	5,1	2	312.5 (M:312.5)	4	29,8	3,11
tr|F0P9S0|F0P9S0_STAPE	Urease accessory protein UreG OS=Staphylococcus pseudintermedius (strain ED99) GN=ureG PE=3 SV=1	22,4	4,9	1	110.8 (M:110.8)	2	12,3	3,91
tr|E8SFZ9|E8SFZ9_STAPH	Urease subunit alpha OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=ureC PE=3 SV=1	61,5	5,2	3	360.2 (M:360.2)	9	18,2	4,21
tr|F0P445|F0P445_STAPE	Urease subunit beta OS=Staphylococcus pseudintermedius (strain ED99) GN=ureB PE=3 SV=1	13,2	5,9	3	130.4 (M:130.4)	2	20,3	6,27
tr|F0P7E2|F0P7E2_STAPE	Uridylate kinase OS=Staphylococcus pseudintermedius (strain ED99) GN=pyrH PE=3 SV=1	26,2	5,4	2	455.4 (M:455.4)	7	31,2	2,96
tr|F0P6T2|F0P6T2_STAPE	Uroporphyrinogen decarboxylase OS=Staphylococcus pseudintermedius (strain ED99) GN=hemE PE=3 SV=1	38,4	5,9	2	203.0 (M:203.0)	4	11,9	5,36
tr|E8SFH0|E8SFH0_STAPH	Uroporphyrinogen-III methyltransferase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_2071 PE=3 SV=1	34,4	5,9	3	178.8 (M:178.8)	4	15,6	1,07
tr|F0P374|F0P374_STAPE	UTP-glucose-1-phosphate uridylyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=galU PE=4 SV=1	32,5	4,9	2	174.3 (M:174.3)	4	13,9	1,95
tr|E8SJ97|E8SJ97_STAPH	Xaa-His dipeptidase OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1518 PE=4 SV=1	52,6	4,6	2	646.2 (M:646.2)	15	37,1	3,47
tr|F0P974|F0P974_STAPE	Xanthine phosphoribosyltransferase OS=Staphylococcus pseudintermedius (strain ED99) GN=xpt PE=3 SV=1	21	4,9	1	109.6 (M:109.6)	3	23,7	265,32

tr|E8SEP3|E8SEP3_STAPH	*YjcG, 2H phosphoesterase superfamily protein OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_0698 PE=4 SV=1*	*19,5*	*4,8*	*2*	*296.8 (M:296.8)*	*6*	*34,5*	*1,51*
tr|F0P366|F0P366_STAPE	*zinc ABC transporter substrate-binding protein*	*62*	*5,4*	*2*	*131.3 (M:131.3)*	*3*	*5,5*	*6,36*
tr|F0P685|F0P685_STAPE	*zinc metallopeptidase*	*25,7*	*9,2*	*1*	*129.4 (M:129.4)*	*3*	*12,5*	*5,79*
tr|F0P469|F0P469_STAPE	*Zinc metalloproteinase aureolysin OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0326 PE=4 SV=1*	*55,1*	*4,7*	*7*	*624.3 (M:624.3)*	*10*	*25,1*	*2,98*
tr|E8SGI0|E8SGI0_STAPH	*Zinc protease OS=Staphylococcus pseudintermedius (strain HKU10-03) GN=SPSINT_1008 PE=3 SV=1*	*49*	*5,2*	*2*	*240.1 (M:240.1)*	*8*	*17,9*	*6,72*
tr|F0P5M9|F0P5M9_STAPE	*Zinc-binding alcohol dehydrogenase OS=Staphylococcus pseudintermedius (strain ED99) GN=SPSE_0119 PE=3 SV=1*	*37,2*	*5,1*	*1*	*488.0 (M:488.0)*	*10*	*28,5*	*5,59*